



# JOURNAL of FOOD SCIENCE

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# Letters TO THE SCIENTIFIC EDITOR

## Letter to the Editor

January 24, 1974

Dear Sir:

I would like to comment on the paper "Effects of end point and oven temperatures on beef roasts cooked in oven film bags and open pans" by T.A. Shaffler, D.L. Harrison and L.L. Anderson [*J. Food Sci.* 38(7): 1205]. I would like to commend the authors for their work in the economically important area of meat processing but would suggest that different conclusions can be drawn from the data presented.

It is a popular misconception that the center temperature of a roast at the termination of cooking is an effective measure of the thermal processing that roast has received. However, if the ambient temperature is significantly higher than this temperature, there will be a large overshoot in the center temperature after the roast has been pulled from the heated environment. The extent of this overshoot will depend on such properties as the roast configuration, size, temperature at the start of cooking, external heat transfer coefficient, fat layer configuration and grain direction. For example, in a classroom demonstration of the use of finite differences to model the cooking process of 6.8 kg spherical roasts initially at 4°C and heated in an oven at 177°C to an internal temperature of 60°C and then pulled to an environment at 20°C, the predicted center temperature continued to climb to 80°C. Of course, the conditions reported in the paper are quite different from those used in the model, but this gives an order of magnitude of the overshoot phenomena. If the cooking conditions of a roast are constant from one roast to the next, the extent of the overshoot will also be constant. If, on the other hand, the conditions change (as by changing from dry roasting to in-the-bag roasting) the *amount of overshoot will also change*. In general, a higher heat transfer coefficient at the surface will cause a steeper heating front profile and thus a higher extent of overshoot.

This seems to be the case in the data given in the article. The use of the bag (thus cutting down evaporation) gives a larger heat flux into the roast at the end of the roasting period. (Interestingly, the bag also slows the heat transfer process during the thawing period since during this period evaporation at the surface of the dry heat method is small and the film adds a significant thermal barrier as shown in the larger thawing times.) The fact that the moist heat samples were overcooked is apparent by the last figures in Tables 1 and 2. The effect of the type of heat on the "apparent degree of doneness" is greater than the effect of the end point temperature. The most obvious explanation is that use of end point center temperatures is inappropriate in comparing the two cooking methods since the same end point center temperature for the moist samples drastically overcooks them compared to the dry heat samples.

One further comment is that the authors' use of the extremely high levels (60–80°C) for end point temperatures should be explained or justified since they would seem to be much higher than appropriate for the roasting of tender cuts of meat.

JOHN ROSENAU  
University of Massachusetts  
Amherst, MA 01002

## Letter to the Editor

February 19, 1974

Dear Dr. Liska:

Thank you for the opportunity to reply to Dr. Rosenau's comments on our paper, "Effects of end point and oven temperatures on beef roasts cooked in oven film bags and open pans." The objective of our experiment was to investigate the effects on beef top round of roasting and cooking in oven film from the frozen state at two oven temperatures (177° and 205°C) and to three end point (internal) temperatures (60°, 70° and 80°C). I believe that we have presented data that show the effects of each method of cookery on the roasts, and that the data support our conclusions. Our methods for collecting data are generally accepted for the type of study we made.

There may be a popular misconception that the center temperature of a roast at the termination of cooking is an effective measure of the thermal processing the roast has received. However, that is not true for professional home economists and meat scientists who have conducted extensive research involving meat cookery. We are aware that heat from the cooking environment is transferred from the outer surface to the center of the roast, and that at any given time in the cooking process, the temperature at the center of a roast is lower than that in other parts. Thus, characteristics of the meat differ in different positions of the roast. That is why servings of rare, medium- and well-done meat may be carved from a large beef roast cooked to an internal end point of 60°C. We have described the internal appearance of roasts cooked by each type of heat to each end point temperature and the surface appearance of roasts cooked by the two types of heat. Dr. Rosenau didn't suggest an alternative for our method of measuring the end point of cooking.

Also, we are aware that the temperature differential between different positions in a roast, the rate heat penetrates the roast, and post-oven temperature rise are affected by the factors listed by Dr. Rosenau. That is why we controlled the weight and configuration of roasts used in our experiment.

There were no statistically significant differences attributable to the weight of roasts assigned to the six treatment-combinations designated as subplots in a splitplot design with oven temperatures being the mainplots (Table 1 and 2, JFS 38: 1206). Fig. 1 (JFS 38: 1205) indicates that the grain of the muscle was considered when roasts were cut.

Anyone who is familiar with the semimembranosus and adductor muscles of bovine top round knows that there are no large fat pockets within those muscles. We removed the outer fat covering from the top rounds before they were cut into roasts.

Dr. Rosenau gave an example of the "overshoot" obtained in a classroom demonstration with a 6.8 kg spherical roast heated at 177°C to 60°C. He recognized that the conditions of his demonstration were different from those in our experiment, but stated that his example gives an order of the magnitude of the overshoot phenomena. It is unreasonable to expect the overshoot to be similar for a 6.8 kg roast and a 1.1 kg roast, the mean weight of roasts in our experiment; the larger the roast, the greater the overshoot expected. Also, in our experience the overshoot may vary considerably within a group of roasts of similar weight and configuration. Dr. Rosenau didn't state whether his example represented data from several replications or from a single demonstration.

We agree with Dr. Rosenau's explanation of why the overshoot for roasts cooked by dry and moist heat would not be expected to be similar. Our data confirm his statement. However, we do not agree that roasts cooked in the bag were overcooked because they appeared more well-done at a given end point temperature than roasts cooked in open pans. Apparent degree of doneness was scored on the basis of the "usual conception" of rare, medium- and well-done beef.

Dr. Rosenau's statement concerning the "extremely high levels (60–80°C) for end point temperatures would seem to be much higher than appropriate for roasting tender cuts of meat" is incorrect. Generally accepted internal end point temperatures for degree of doneness of beef are: 60°C (140°F), rare; 70°C (158°F), medium-done; and 76–80°C (170–176°F), well-done. For safety, I wouldn't serve to a sensory panel meat cooked to an internal temperature of less than 60°C. Beef top round generally is not considered a "tender" cut. It may be roasted in an open pan, because there is enough moisture in the muscle itself to create partial hydrolysis of collagen, and thus, softening of collagenous connective tissue when heated.

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# ABSTRACTS:

## IN THIS ISSUE

**PASSION FRUIT STARCH AND EFFECT ON JUICE VISCOSITY.** S.C.M. KWOK, H.T. CHAN JR., T.O.M. NAKAYAMA & J.E. BREKKE. *J. Food Sci.* 39, 431–433 (1974)—Starches in the juices of yellow passion fruit, *Passiflora edulis* f. *flavicarpa*, and purple passion fruit, *P. edulis* f. *edulis*, were isolated and characterized. Starch granule sizes for the yellow variety (7.8  $\mu$ m) and purple variety (6.4  $\mu$ m) were similar. Gelation temperature ranges for the yellow variety (58.5–67.0°C) and purple variety (58.5–66.5°C) were also similar. The amylose content was slightly higher in the yellow variety (8.7%) than in the purple variety (5.8%). Viscosity differences between juices of the two varieties after treatment with heat and  $\alpha$ -amylase were attributed to differences in pH and starch content between the yellow variety (pH 2.8, 0.06% starch) and the purple variety (pH 4.2, 0.74% starch).  $\alpha$ -amylase was effective in reducing the viscosity of passion fruit juice in which the starches are gelatinized.

**DETERMINATION OF VOLATILES BY VAPOR HEADSPACE ANALYSIS IN A MULTI-PHASE SYSTEM: d-LIMONENE IN ORANGE JUICE.** H.A. MASSALDI & C.J. KING. *J. Food Sci.* 39, 434–437 (1974)—Headspace analysis of volatile compounds is complicated when components are present above their limit of solubility and/or when a second solid or liquid phase exists. A technique is presented which is based upon controlled dilution to a concentration where the solubility limit is no longer exceeded, coupled with a correction for extraction into the additional solid or liquid phase(s). The method is demonstrated for the determination of d-limonene contents of synthetic emulsions and orange juice.

**VOLATILES RETENTION DURING FREEZE DRYING OF SYNTHETIC EMULSIONS.** H.A. MASSALDI & C.J. KING. *J. Food Sci.* 39, 438–444 (1974)—Experimental results are presented for the retention of d-limonene during freeze drying of sucrose solutions, for 10% and 25% solution concentrations, for fast and slow freezing, and for various levels of d-limonene addition ranging from below to considerably above the solubility limit. The results show decreasing percentage retentions with increasing initial levels of d-limonene, reaching an asymptotic value at high initial d-limonene contents. These results are interpreted quantitatively in terms of both microscopic observations and a simple model which allows for the collection of emulsion droplets at the ice-concentrate interface during the freezing step, with subsequent loss of those droplets during freeze drying. Retention experiments with n-hexyl acetate and acetone lend additional support to this interpretation and demonstrate the importance of the ratio of emulsion droplet size to the thickness of the interstitial webs of concentrate.

**RETENTION OF D-LIMONENE DURING FREEZE DRYING OF ORANGE JUICE.** H.A. MASSALDI & C.J. KING. *J. Food Sci.* 39, 445–448 (1974)—Retention of d-limonene during freeze drying of citrus juices from several sources was investigated as a multi-phase problem. Reconstituted to 25 and 10° Brix, commercial frozen concentrates show high retention (between 90 and 100%), whereas fresh-squeezed juice shows a relatively low retention (about 30%), comparable to that found previously for synthetic emulsions. Retention data for pasteurized fresh juice after substantial agitation and for never-concentrated, commercial pasteurized juices show an intermediate value (between 60 and 70%). Presence and stability of cloud are shown to correlate closely with improved d-limonene retention in commercial frozen concentrates. On this basis, a simple model is developed which predicts the retention level for this type of juice in the asymptotic region. The results for the other types of juice studied are interpreted with the aid of microscopic determinations of oil droplet size and the model derived for synthetic emulsions.

**ANTHOCYANINS OF BEAUTY SEEDLESS GRAPES.** T. PHILIP. *J. Food Sci.* 39, 449–451 (1974)—Beauty Seedless grape pigments were isolated and identified by chromatographic, spectral and chemical properties. The pigments were identified as delphinidin-3-glucoside, petunidin-3-glucoside, malvidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside acylated with caffeic acid and malvidin-3-glucoside acylated with p-coumaric acid. Beauty Seedless grapes contained 62 mg anthocyanins per 100g fresh grapes and malvidin derivatives accounted for 73% of the total anthocyanins.

**ETHYLENE INDUCED ISOPEROXIDASE CHANGES DURING FIBER FORMATION IN POSTHARVEST ASPARAGUS.** N.F. HAARD, S.C. SHARMA, R. WOLFE & C. FRENKEL. *J. Food Sci.* 39, 452–456 (1974)—Excision of asparagus spears triggered increased peroxidase activity and a shift in isozyme species. Changes in peroxidase occurred within 8 hr after harvest at the cut end of the spear and progressed to the tip end after 24 hr storage at 20°C and 85% relative humidity. Isozyme changes were accelerated by exogenous ethylene gas. Ethylene production by cut spears and rate of spear toughening were greatest when spears were cut above ground. It is suggested that wound ethylene triggers a shift in peroxidase isozymes which participate in lignin biosynthesis and results in spear toughening after harvest.

**KINETICS OF THE THERMAL DEGRADATION OF METHYLMETHIONINE SULFONIUM IONS IN CITRATE BUFFERS AND IN SWEET CORN AND TOMATO SERUM.** M.P. WILLIAMS & P.E. NELSON. *J. Food Sci.* 39, 457–460 (1974)—Thermal degradation of methylmethionine sulfonium ions was determined in sodium citrate buffers ranging in pH from 4.0–8.0. Reaction temperatures of 82°, 88°, 93° and 99°C were studied at reaction times of 20, 40, 60 and 80 min. The same conditions were used to study the degradation of naturally occurring methylmethionine sulfonium ions in sweet corn and tomato serums. In each solvent system, the thermal degradation of these salts occurred according to first order kinetics; the reaction rates doubled for a 5–6°C increase in temperature and activation energies of the reaction in the various media were comparable. The characteristic degradation kinetics provides a means of identifying methylmethionine sulfonium salts in fresh foods without having to resort to involved isolation procedures.

**FUNCTIONAL CHARACTERISTICS OF MUSTARD MUCILAGE.** F.E. WEBER, S.A. TAILLIE & K.R. STAUFFER. *J. Food Sci.* 39, 461–466 (1974)—Mucilage found in hulls of yellow mustard seed was investigated. The isolated mucilage was a cold water-swelling hydrocolloid of low to medium viscosity and exhibited thixotropic properties. Viscosity increased with addition of alkali or acid. Mucilage produced synergistic increases in viscosity when combined with guar, locust bean, or carboxymethylcellulose. Solutions were opaque, bland tasting and slightly acidic. Solutions showed stability to acidity down to pH 3.5 and also to cooking and cooling cycles. The mucilage lowered the surface tension of water and the interfacial tensions of oil and water emulsions. Emulsification was achieved in model salad dressing formulations. The mucilage exhibited excellent suspending properties.

**SOME PHYSICO-CHEMICAL PROPERTIES OF STARCHES AS AFFECTED BY CHANGES IN ATMOSPHERIC PRESSURE.** L. MORROW & K. LORENZ. *J. Food Sci.* 39, 467–470 (1974)—The effects of varying atmospheric pressures on the properties of starches were investigated.

Four types of starches—corn, rice, potato and wheat—were studied for their water-binding capacity and viscosity behavior. Photomicrographs of starch suspensions were taken at five altitudes and temperatures of 22°, 60°, 70°, 80° and 90°C, respectively. Measurements of the photomicrographs showed corn, potato and rice starch granules to increase in size on changing elevation from sea level to 10,000 feet. Wheat starch was not affected. Water-binding capacity increased for corn and potato starches as the altitude increased, but rice and wheat starches were not affected. The viscosity of corn, rice and wheat starch suspensions decreased initially with increasing altitudes but increased at the higher altitudes. The viscosity of potato starch suspensions did not change at increased elevations.

**CEREBROSIDE IN RICE GRAIN.** Y. FUJINO, S. SAKATA & M. NAKANO. *J. Food Sci.* 39, 471–473 (1974)—Cerebroside (hexosylceramide) was isolated from rice grain, identified and examined for composition of fatty acid, sugar and long-chain base constituents. The cerebroside was observed to contain 12 fatty acids; hydroxyarachidic (59.0%), hydroxylignoceric (19.7%) and hydroxybehenic (10.3%) acids were predominant. Glucose was the only sugar constituent. Three component long-chain bases were found: two were identified as sphingosine (72.9%) and dehydrophytosphingosine (17.3%). It is thus suggested that the major species of rice grain cerebroside is N-hydroxyarachidyl-glucosyl-sphingosine.

**FORMATION OF N-NITROSAMINES IN LOW MOISTURE SYSTEMS.** J.I. GRAY & L.R. DUGAN JR. *J. Food Sci.* 39, 474–478 (1974)—Experiments have shown that N-nitrosamines can be formed when sodium nitrite and dialkylamines are heated in low-moisture model systems. Parameters which may influence the reaction were investigated. Results revealed that maximum N-nitrosamine formation depended on temperature and time of heating, sodium nitrite-amine ratio and pH of the system.

**ANTIOXIDANT EFFECTIVENESS IN INTERMEDIATE MOISTURE CONTENT MODEL SYSTEMS.** H.E. CHOU & T.P. LABUZA. *J. Food Sci.* 39, 479–483 (1974)—The effectiveness of antioxidants was studied in methyl linoleate-carbohydrate model systems at the intermediate moisture content. Systems showing sorption hysteresis were used in the range of  $A_w$  0.68–0.84. Metal chelating agents were found to be more effective than free radical chain terminators. The antioxidant effectiveness of EDTA (a chelator) was greater in the higher moisture desorption systems since more would be expected to be in solution. The work also showed that metal catalyst activity is responsible for the rapid oxidation rates in intermediate moisture systems as compared to dry systems.

**ON THE NATURE OF THE CHEMICAL AND PHYSICAL BONDS WHICH CONTRIBUTE TO SOME STRUCTURAL PROPERTIES OF PROTEIN FOODS: A HYPOTHESIS.** H. BUTTKUS. *J. Food Sci.* 39, 484–489 (1974)—Extractability of the contractile proteins of muscle with salt solutions decreases during freezer storage or on heating. Studies on the insolubilized proteins with dissociating-reducing solutions containing guanidine HCl (GuHCl) and mercaptoethanol or sodium borohydride ( $\text{NaBH}_4$ ) have previously suggested that the mechanisms of myosin aggregation in solution involve noncovalent interactions of hydrophobic and hydrogen bonds as well as the formation of disulfide (S-S) bonds between peptide chains of different molecules. Further evidence is now presented which implies that these types of denaturation mechanisms are of a more general nature and are also responsible in the hardening, firming or gelling of the protein structures of egg white, kamaboko and meats from fish and mammals during cooking. Methods are described by which foods whose structures are dependent upon these types of bonds can be dissolved nonhydrolytically, i.e., without excessive breaking of peptide chains and the destruction or racemization of amino acids which occur during acid or alkaline treatment at elevated temperatures.

**ESTIMATION OF CHANGES IN THE AVAILABILITY OF EACH INDIVIDUAL ESSENTIAL AMINO ACID IN FOOD PROTEINS.** M. WOMACK, C.E. BODWELL & D.A. VAUGHAN. *J. Food Sci.* 39,

490–493 (1974)—A technique has been developed for determining decreases in the availability of individual essential amino acids in a food as a result of processing, cooking or other treatments. Diets containing casein and free L-amino acids were used to establish critical dietary amino acid levels. These critical levels were such that reductions of 20% in the level of any of nine essential amino acids significantly reduced protein utilization as estimated by rat bioassays. When these critical levels were used in evaluating amino acid availability in unheated and heated lactalbumin, each of the nine essential amino acids was found to be affected by the heat treatment.

**INFLUENCE OF MOIST-HEAT TREATMENTS OF PEANUTS ON PEANUT PASTE CHARACTERISTICS.** K.H. McWATTERS & E.K. HEATON. *J. Food Sci.* 39, 494–497 (1974)—Further information on the effects of moist heat on certain qualities of peanuts was obtained by heating skin-free kernels in water at 90, 120, 150, 180 and 210°F for 15, 30, 45, 60, 75 and 90 min. They were drained, held overnight and ground into paste, and evaluated for moisture uptake, shear resistance, color, grinding characteristics and flavor. The performance of peanut paste in a food system was determined by substituting it for almond paste in a macaroon cookie formula. Results showed that moisture uptake and L and b color values increased as the heating temperature and time increased. Resistance to shear decreased as heating time increased for samples heated at 150, 180 and 210°F. At 90 and 120°F, however, there were irregular patterns of moisture uptake and shear resistance. All samples were easily ground in a stone mill except those heated at 120°F for 75 and 90 min which were sticky and gummy. Sensory evaluation indicated that the peanut flavor of the paste was improved by heating at temperatures of 180 and 210°F. Both heating time and temperature affected the consistency and handling of macaroon cookie batters prepared from the paste as well as the sensory qualities of the finished products. Cookies having the highest overall quality were obtained from pastes with a moisture content of 23–27% and heat treatments of 30 or 45 min at 180°F or 15 min at 210°F.

**PREPARATION AND CHARACTERIZATION OF  $\alpha$ -AMYLASE IMMOBILIZED ON COLLAGEN MEMBRANES.** D.H. STRUMEYER, A. CONSTANTINIDES & J. FREUDENBERGER. *J. Food Sci.* 39, 498–502 (1974)—Crystalline pancreatic  $\alpha$ -amylase was codispersed with hide collagen at pH 4.0 and tanned to form a membrane which degraded starch. The optimum pH for the codispersed membrane preparation was at pH 7.0 in contrast to the soluble enzyme which was active at pH 8.0 as at pH 7.0. The immobilized enzyme responded maximally to 0.22M chloride whereas 0.02M chloride gave optimum rates for the soluble enzyme. The immobilized enzyme resisted thermal inactivation better than the soluble  $\alpha$ -amylase. Raising the temperatures from 30° to 50°C produced a 500% increase in rate for the bound enzyme. It was also demonstrated that membranes retained greater activity when stored in starch solution than in water. The effect of glutaraldehyde concentration on membrane activity was also studied.

**REPLICATED COMPOSITE COMPLETE-INCOMPLETE BLOCK DESIGNS.** J.A. CORNELL & F.W. KNAPP. *J. Food Sci.* 39, 503–507 (1974)—Composite complete-incomplete (C-I) block designs are formed by combining complete blocks of size t units with balanced incomplete blocks of k units ( $1 \leq k < t$ ) resulting in blocks of size t+k units. In sensory experiments, composite C-I block designs have been shown to be more efficient than the standard complete block designs. Replication of the blocks in these C-I block designs permits accurate interpretation of panelist  $\times$  treatment interaction effects. Also, the formulae for calculating estimates of the treatments, panelists, and interaction effects are simpler in form with the replicated C-I designs than the corresponding formulae with the unreplicated C-I block designs. The analysis of an experiment involving the rating of flavor of three strawberry preserves using a replicated C-I block design is presented.

**REFLECTANCE COLORIMETRIC EVALUATION OF EGG YOLK PIGMENTATION.** J.L. FRY, C.F. HINTON & R.H. HARMS. *J. Food Sci.* 39, 508–510 (1974)—Moderately pigmented yolks were diluted with pigment-free yolks to provide a wide range of samples with which to

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compare standard beta carotene equivalents with excitation purity and luminous reflectance values obtained with a reflectance colorimeter. Pigmented yolks were also mixed with a white diluent for reflectance colorimetric comparisons. Correlation coefficients of beta carotene equivalents with excitation purity and luminous reflectance values were +0.906 and -0.874, respectively. The regression line for luminous reflectance was linear and for excitation purity was curvilinear. The white diluent (1:1 ratio) resulted in significant reduction of the tendency of excitation purity to plateau for deeply pigmented yolks.

**CATHEPTIC ENZYME ACTIVITY IN AGED COUNTRY-STYLE HAMS AS INFLUENCED BY PRE-CURING TREATMENTS.** T.S. MELO, T.N. BLUMER, H.E. SWAISGOOD & R.J. MONROE. *J. Food Sci.* 39, 511-515 (1974)—Chemical composition and catheptic activity were determined in fresh chilled hams and four lots of cured hams subjected to different pre-curing treatments. Hams were sampled after aging (storage) of 2, 4 and 6 months at 29°C. Moisture decreased, while protein, salt and free amino acid concentrations increased with aging time of cured hams, but treatment effects were similar. Frozen, thawed cured hams aged for 2 months exhibited the highest specific enzyme activity but decreased in enzyme activity after 4 and 6 months of aging. Free amino acid concentration after aging was higher in hams frozen 1-3 yr ( $\bar{x}$  = 2.3) at -29°C than for those frozen 0 or 2 days.

**FACTORS RESPONSIBLE FOR WHITE FILM FORMATION ON CUT SURFACES OF DRY-CURED HAMS.** R.G. BUTZ, T.N. BLUMER, J.A. CHRISTIAN, H.E. SWAISGOOD & H.L. LUCAS. *J. Food Sci.* 39, 516-519 (1974)—White surface film that forms on cut surfaces of country-style ham was identified as crystalline L-tyrosine. Free L-tyrosine averaged 287.3  $\mu$ moles/g (5.2% by weight) in the film and 6.1  $\mu$ moles/g in ham tissue from which film had been removed. It is concluded that the film forms because the concentration of free L-tyrosine and certain other free amino acids in ham is greater than solubility levels; therefore, crystals form on the cut surface. The absence of surface film on knuckle muscles and film formation on other muscles of the same slice was attributed to higher NaCl ( $P < 0.05$ ) of the former which decreased catheptic activity.

**COMPARISON OF SALAMI SAUSAGE PRODUCED WITH AND WITHOUT ADDITION OF SODIUM NITRITE AND SODIUM NITRATE.** R. SKJELKVÅLE, T.B. TJABERG & M. VALLAND. *J. Food Sci.* 39, 520-524 (1974)—A pilot plant production of salami dry sausage with and without the addition of nitrite was carried out. The microbiological, rheological and organoleptic properties of the products were investigated during the fermentation process and after storing the products at 20°C for 3 months. Results of the microbiological investigation indicate that the fermentation and ripening of the sausage would take a normal course without the addition of nitrite, glucono-delta-lactone (GDL) or starter culture. In a triangle test performed at the end of the ripening period, the taste panel was unable to distinguish between products with and without nitrite. After 3 months storage a significant difference was found between products with and without the addition of nitrite, and the nitrite-cured products were given the best score. In series in which GDL or starter culture was added, no significant differences in organoleptic quality were found even after storage for 3 months.

**ISOMETRIC TENSION STUDIES ON CHICKEN PECTORALIS MAJOR MUSCLE.** D.F. WOOD & J.F. RICHARDS. *J. Food Sci.* 39, 525-529 (1974)—Broiler Pectoralis major developed and released isometric tension

in phosphate buffer at varying temperatures and pH levels. The presence of 1 mM calcium or magnesium in the incubation buffer enhanced tension release as did EDTA and combinations of calcium-EDTA or magnesium-EDTA. Tension release rate varied among birds being fastest in those with the shortest time to maximum tension. Amount of tension released 2 hr post-maximum tension correlated significantly ( $P < 0.01$ ) with amount released from 3 through 12 hr. 2-hr values correlated negatively ( $P < 0.01$ ) with time to maximum tension but not tenderness (shear value), suggesting that amount of tension release is not indicative of tenderness differences among birds.

**COLD SHORTENING IN CHICKEN BROILER PECTORALIS MAJOR.** D.F. WOOD & J.F. RICHARDS. *J. Food Sci.* 39, 530-531 (1974)—At 2°C strips of P. major muscle from chicken broilers developed a peak in isometric tension which quickly dissipated and was followed by no subsequent changes in tension during the next 36 hr. Increasing the temperature at which strips were maintained from 2° to 23°C (22°-25°C) after 12 or 24 hr resulted in some further tension development but essentially no tension development was observed in strips which had been held at 2°C for 36 hr before the temperature was raised to 23°C. Cold shortening did not significantly lower muscle ATP, creatine phosphate or hexose monophosphate levels.

**CHANGES IN TENDERNESS OF BEEF LONGISSIMUS DORSI AS RELATED TO MUSCLE COLOR AND pH.** H.T. FREDEEN, A.H. MARTIN & G.M. WEISS. *J. Food Sci.* 39, 532-536 (1974)—Longissimus dorsi shear values for carcasses of 158 steers, 147 heifers and 259 bulls, all slaughtered in the age range of 12-16 months, were correlated with postmortem pH values and postmortem change in pH. Neither the individual pH values nor degree of postmortem pH change were useful predictors of shear value for steer and heifer carcass ( $r^2$  values less than 0.07). For bull carcasses, pH of the longissimus dorsi taken 24 hr post-mortem was significantly correlated with shear value, ( $r = -0.7$ ) and with meat color ( $r = +0.7$ ). Sexes differed markedly in the range of 24 hr pH of the longissimus dorsi with none of the steers, 8% of the heifers and 49% of the bulls having values greater than 6.0.

**THE AROMA OF CANNED BEEF: APPLICATION OF REGRESSION MODELS RELATING SENSORY AND CHEMICAL DATA.** T. PERSOON & E. von SYDOW. *J. Food Sci.* 39, 537-541 (1974)—The purpose of this investigation was to determine general relations between instrumental and sensory aroma data from a reference material consisting of a large set of different types of beef samples analyzed during several years. The relations obtained in this way have been tested on independent "unknown" samples. Different models have been used, basically derived from Stevens' law and formulated in analogy with models used in other psychophysical contexts. From the reference material a great number of highly significant relations—several with a correlation coefficient greater than 0.90—were obtained for the various odor notes used. Several of these seem to be examples of causative relations. When predicting sensory properties of unknown samples almost all the relations obtained with high correlation coefficient worked very well. These properties could be predicted by the gas chromatographic technique with the same accuracy as when the panel assessed the samples. Therefore, by applying the models in a proper way, the panel service in routine analyses may be supplemented or refined by using a gas chromatographic technique. These methods may, of course, also be used in product and process development work.



**PRESSURE-INDUCED SOLUBILIZATION OF MEAT PROTEINS IN SALINE SOLUTION.** J.J. MACFARLANE. *J. Food Sci.* 39, 542–547 (1974)—Under some conditions, pressures of the order of 100 meganewtons/meter<sup>2</sup> (MNm<sup>-2</sup>) applied to homogenates of ovine meat in 0.5M sodium chloride solution were found to give about a threefold increase in the yield of salt-soluble protein. At pressures of about 70 MNm<sup>-2</sup> the water-holding capacity (WHC) of homogenates of meat from some carcasses was increased. The effect of pressure on protein solubility was dependent on meat concentration in the homogenate (pressure was ineffective at high meat concentrations); duration of pressurization; and pH (being most effective in the pH range 5.3–7). The pressure-induced solubilization of meat proteins is assumed to occur via an ionic mechanism.

**MUSCLE CATHEPSINS IN THREE SPECIES OF PACIFIC SOLE.** G.M. GEIST & D.L. CRAWFORD. *J. Food Sci.* 39, 548–551 (1974)—The cathepsins in saline muscle extracts of three species of Pacific sole were investigated. A buffered hemoglobin solution (pH 3.0) served as substrate for the enzyme assay. No apparent change in catheptic activity in muscle homogenates of English sole was observed during 25 days of frozen (–26°C) storage. Partial characterization of the cathepsins indicated a pH activity optimum of 3.0–3.5 for rex and petrale sole but a slightly higher pH optimum of 3.2–3.8 for English sole. The three species showed a temperature optimum of 45°C for hemoglobin splitting. A wide variation in catheptic activity within the species was observed for rex sole while English and petrale sole showed more uniform catheptic activity. The mean activities for the three species varied significantly ( $P < 0.05$ ). Results of sensory evaluation of muscles containing high and low levels of catheptic activity from the three species of sole did not support the assumption that cathepsins have a substantial influence upon the quality of marine food fish.

**IRON FORTIFICATION OF DEHYDRATED MASHED POTATOES.** G.M. SAPERS, O. PANASIUK, S.B. JONES, E.B. KALAN, F.B. TALLEY & R.L. SHAW. *J. Food Sci.* 39, 552–554 (1974)—Experiments were carried out to determine the feasibility of the fortification of dehydrated mashed potatoes with iron at levels as high as 10 mg/100g. Iron compounds were screened in fresh and dehydrated mashed potatoes to determine the extent of iron-induced discoloration. The flavor and stability of fortified potato flakes were evaluated by sensory and GLC procedures. Discoloration occurred in most fortified samples and appeared to parallel the biological availability of the iron compound being tested. Potato flakes fortified with two ferripolyphosphate complexes were less stable during storage than unfortified controls, developing off-flavors and high levels of volatile oxidation products. Iron fortification of dehydrated mashed potatoes as reported herein is not considered feasible.

**FLAVOR QUALITY AND STABILITY OF POTATO FLAKES: EFFECTS OF DRYING CONDITIONS, MOISTURE CONTENT AND PACKAGING.** G.M. SAPERS, O. PANASIUK, F.B. TALLEY & R.L. SHAW. *J. Food Sci.* 39, 555–558 (1974)—Dehydrated potato flakes were evaluated by sensory and GLC procedures to determine the effects of packaging and drying conditions on flavor quality and stability after storage in air. Stability was not affected by the choice of metal vs. polyethylene as the packaging material or by the package headspace volume. Comparisons of flakes drum dried at different rates and to different moisture contents indicated that overdrying reduced flake stability due to thermal damage during dehydration and to the low water activity of the over-dried product. Mash adhering to drum-drier applicator rolls is a potential source of potato flake instability, especially, if raw material quality is poor.

**PREPARATION AND STORAGE PROPERTIES OF DRUM DRIED WHITE YAM (*Dioscorea rotundata* Poir) FLAKES.** O. ONAYEMI & N.N. POTTER. *J. Food Sci.* 39, 559–562 (1974)—The white yam *Dioscorea rotundata* Poir was characterized, drum dried to different moisture levels, and stored at temperatures of 21.1°C, 29.4°C and 37.8°C for 90 days. Fresh and stored samples were analyzed for changes in color, ascorbic acid and organoleptic qualities. Freshly dried and reconstituted yam flakes were judged highly acceptable compared to conventionally

mashed fresh yam and retained acceptability through storage. The taste panel consisted of persons from several West African countries. Changes in the flakes during storage are described.

**COLOR OF PROCESSED SWEET POTATOES: EFFECTS OF CAN TYPE.** L.E. SCOTT, B.A. TWIGG & J.C. BOUWKAMP. *J. Food Sci.* 39, 563–564 (1974)—Sweet potatoes packed in four types of enamel-lined cans were darker in color and less attractive than those packed in tin-coated cans. The differences in color among can types was more pronounced after opening the canned product and exposure to air for an hour. The tin content of the product from the tin-coated cans was much greater than that from the enamel-lined cans. The brighter color of the product from the tin-coated cans is assumed to result from the substitution of tin for iron or other metals in the complex formed in the phenolase reactions.

**COLOR OF PROCESSED SWEET POTATOES: EFFECT OF ADDITIVES.** B.A. TWIGG, L.E. SCOTT & J.C. BOUWKAMP. *J. Food Sci.* 39, 565–567 (1974)—The additives, ethylenediaminetetraacetic acid (EDTA), stannous chloride, citric acid, ascorbic acid and sodium bisulphite were added to syrup and vacuum packs of sweet potatoes. Addition of EDTA, SnCl<sub>2</sub>, citric acid and sodium bisulphite was effective in decreasing darkening of the processed product. SnCl<sub>2</sub>, EDTA and citric acid were also effective in preventing discoloration after exposure of the product to air. The action of the additives is attributed to a disruption of the reaction leading from oxidation of polyphenols to the production of dark-colored compounds.

**APPLICATION AND ADVANTAGES OF THE ENZYMATIC METHOD FOR THE ASSAY OF ASCORBIC AND DEHYDROASCORBIC ACIDS AND REDUCTONES.** Determination in Fresh and Canned Spinach. A. MARCHESINI, F. MONTUORI, D. MUFFATO & D. MAESTRI. *J. Food Sci.* 39, 568–571 (1974)—A new enzymatic method based on the reaction ascorbic acid + ½O<sub>2</sub> → dehydroascorbic acid + H<sub>2</sub>O permits assaying for ascorbic and dehydroascorbic acids in vegetable extracts. The method is carried out using a Clark's electrode which determines the oxygen uptake during the above reaction. The method applied to both fresh and canned spinach is specific and reproducible. Some interfering substances may reduce the specificity and precision of the method, but the addition of chelating agents or acetaldehyde eliminates these interferences. On adding boric acid, only the interfering reductic acid is assayed. The ascorbic and dehydroascorbic acid values for fresh spinach range between 528–919 mg/100g for ascorbic acid and from 21–81 mg/100g for dehydroascorbic acid. For canned spinach the ascorbic acid values ranged from 119–202 mg/100g and the dehydroascorbic acid values from 25–110 mg/100g. The method is easy and rapid and can be utilized advantageously for routine analyses.

**EXTRUSION PROCESSING OF TRITICALE.** K. LORENZ, J. WELSH, R. NORMANN, G. BEETNER & A. FREY. *J. Food Sci.* 39, 572–576 (1974)—Whole triticale kernels were extruded using a Brabender Plastimeter extruder with 3/4-in. rifled barrel and 1:1 flight depth ratio screw. Samples were extruded at initial moisture contents of 15% and 20% using barrel temperatures of 350°F, 400°F and 450°F through nozzle openings of 1/8 in. and 1/16 in. The products were measured for pH, final moisture content, color, trypsin hydrolysis, torque during extrusion, and texture and flavor as evaluated by a taste panel. From the results it was concluded that an acceptable product could be produced at 20% initial moisture using either the small nozzle and operating at 350°F or the larger nozzle and operating at 400°F.

**WHOLE SOYBEANS AS A MEANS OF INCREASING PROTEIN AND CALORIES IN MAIZE-BASED DIETS.** R. BRESSANI, B. MURILLO & L.G. ELÍAS. *J. Food Sci.* 39, 577–580 (1974)—Studies have indicated that one factor responsible for protein-calorie malnutrition in children is the deficiency of good-quality protein and calories in their habitual diets. Previous investigations attempted to solve the problem by means of supplementation with good-quality protein, but this research has not

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considered direct correction of the calorie deficit. Thus studies were carried out to improve both the protein quality and calorie content with a single food: soybean, added and processed together with maize. Two types of food preparations were studied: tortillas made from 85% maize and 15% whole soybeans (processed together by the lime-cooking treatment used for maize) which when compared to the usual one, had a higher protein and calorie content, and was very acceptable in both appearance and taste. Higher levels of soybean mixed with maize were also tested to develop food preparations with higher concentrations of both calories and protein. These were also processed by the lime-cooking treatment. From these tests a mixture of soybean and maize in the proportion 28g soybean to 72g maize gave a food preparation with 18% protein, 10% fat and a high-protein quality as measured by its PER. The use of higher soybean levels did not improve protein quality, and the yields of the resultant products decreased.

**NUTRITIVE VALUE OF A FIBER-FREE COCONUT PROTEIN EXTRACT OBTAINED BY AN ENZYMIC-CHEMICAL METHOD.** P.A. LACHANCE & M.R. MOLINA. *J. Food Sci.* 39, 581-584 (1974)—The fiber-free coconut extract obtained by an enzymic-chemical method (*J. Food Sci.* 38: 607) proved to have a higher protein nutritive value than that of the original coconut meal. The improvement is attributable in part to the fact that the fiber of unmodified coconut meal has an unfavorable effect on the nutritive value of coconut protein. Amino acid analysis revealed that the extracted protein had a higher total essential amino acid content including a higher lysine content and a higher lysine-to-arginine ratio than the original coconut meal.

**USE OF ANIMAL BLOOD AND CHEESE WHEY IN BREAD: NUTRITIVE VALUE AND ACCEPTANCE.** R.P. BATES, L.C. WU & B. MURPHY. *J. Food Sci.* 39, 585-587 (1974)—Breads in which water and milk powder were replaced with unrefined pork blood and/or cottage cheese whey were compared with commercial white bread and an "organic" whole wheat bread. Loaves were evaluated for appearance, composition and acceptance. Rat feeding studies in which the breads contributed about 10% protein to otherwise complete diets indicated no significant differences in growth performance (adjusted PER's) between commercial and "organic" bread. Significantly better performance was obtained for loaves containing blood, whey and blood:whey mixtures. The experimental loaves were comparable in acceptability to commercial breads, although loaf volumes were much lower and crumb texture coarser.

**BONE PARTICLE CONTENT OF SOME MINCED FISH MUSCLE PRODUCTS.** M. PATASHNIK, G. KUDO & D. MIYAUCHI. *J. Food Sci.* 39, 588-591 (1974)—Minced fish muscle recovered by flesh separators may vary in bone particle content depending on the type of machine and the size of the openings that the fish are passed through to screen out the skin and bones. Orderly marketing of these materials will require some measure of control of the bone particle content. The method presented here for quantifying the bone particle content of minced flesh by gravity-flotation involves (1) shredding the flesh with a low-speed stirring device and (2) gravity separation of the bone, cartilage and other high-density components from the lower-density floatable muscle fibers. Experimental samples obtained by passing various fish materials through flesh separators and then through 1- and 2-mm flesh strainers were analyzed for their bone particle content by the proposed method. The bone particle content of flesh containing 25 to more than 100 particles per pound could be reduced by 70-100% by passage through strainers. Imported commercial samples displayed bone particle contents of 4 to

over 400 per pound. Sensory panels could not detect more than 10% of the particles found by objective analysis. The gravity-flotation method may serve as a useful quality control tool.

**SQUID PROTEIN ISOLATE: EFFECT OF PROCESSING CONDITIONS ON RECOVERY YIELDS.** L.N. KAHN, Z. BERK, E.R. PARISER, S.A. GOLDBLITH & J.M. FLINK. *J. Food Sci.* 39, 592-595 (1974)—*Loligo* species of squid was investigated as a potential source of protein isolate. The various process parameters which influence extraction of protein (particle size, time, extraction pH, salt concentration, relative amount of solvent to squid tissue and temperature) were investigated. From this study the following parameters were chosen to optimize extraction: pH 11 (sodium hydroxide) or 4% salt concentration (sodium chloride, sodium hexametaphosphate in aqueous extractant); temperature = 22°C; time = 45 min; particle diameter = 2-3 mm; solvent-to-squid ratio = 10:1. Under these conditions, about 85% of the squid protein can be extracted. 65% of the extracted nitrogen is recovered as protein isolate by isoelectric precipitation at pH 5.

**EFFECT OF SMOKING TEMPERATURES ON ACCEPTABILITY AND STORAGE STABILITY OF SMOKED SPANISH MACKEREL.** J. DENG, R.T. TOLEDO & D.A. LILLARD. *J. Food Sci.* 39, 596-601 (1974)—The effect of temperature and smoking schedules on the quality of Spanish mackerel was evaluated using a pilot plant model of a commercial smokehouse system. Although the final moisture contents of the products are the same, the product smoked only at high temperature (160°F) had the softest texture. Smoking only at low temperature (100°F) did not develop surface color thus resulting in a product having the poorest appearance. A process which combined smoking at both low and high temperature at 60% RH gave a product having excellent texture and appearance. High temperature smoking above 160°F darkened the surface excessively and reduced product acceptability. A holding period after brining and between the cold and hot smoking stages in the process improved appearance and smoke flavor. Water phase salt content judged to be the optimum saltiness was 3.7%. Microbiological counts in the product of the combination cold and hot smoking process were very low (< 10/g). Smoking at 90-100°F did not allow an increase in surface microbiological count. The final step in the process when carried out at 160°F inactivated most of the vegetative microorganisms inoculated on the surface. Storage life of Spanish mackerel produced by cold followed by hot smoking (160°F) was 45 days at 38°F and at least 22 wk at 10°F. This process does not meet the 30 min at 180°F minimum temperature requirement in the Good Manufacturing Practice Regulation of the Food and Drug Administration for smoked fish; therefore, frozen storage and distribution are recommended.

**EFFECT OF SMOKEHOUSE TEMPERATURE, HUMIDITY AND AIR VELOCITY ON RATE OF HEATING AND QUALITY OF FRANKFURTERS.** C.W. MONAGLE, R.T. TOLEDO & R.L. SAFFLE. *J. Food Sci.* 39, 602-604 (1974)—The effects of air velocity, relative humidity, temperature and presence of glucono delta lactone (GDL) on the quality of frankfurters, were evaluated. Rate of heating was inversely proportional to rate of weight loss during processing. High humidity processes at slow air flows had the fastest heating rates; however, quality was unacceptable because of fat separation and pale color. A process where the smokehouse temperature was slowly increased by 6°C increments, and where a low relative humidity and high air flow were used, gave the best quality. Color and texture was more desirable on products having higher shrink. The presence of GDL had no effect on the final color of products processed under the same conditions; however, maximum color appeared to develop earlier in samples containing GDL.

**ACCELERATED PORK PROCESSING: A QUANTITATIVE STUDY OF BACTERIAL FLORA OF CURED AND SMOKED HAMS.** D.G. CORNISH & R.W. MANDIGO. *J. Food Sci.* 39, 605–606 (1974)—96 hams (48 left and right pairs) were assigned to either accelerated or conventional processing methods following separation from their respective sides at a commercial slaughter plant. Bacteriological survey samples were aseptically removed from the accelerated and the conventionally processed hams prior to curing. A second bacteriological sample was removed from both the rapid and conventional treatments following curing, smoking and chilling. Total bacterial counts were then determined on both the fresh and cured muscle tissue. The effect of different processing techniques was evaluated by bacterial flora present in the rapid and conventionally processed hams. Aerobic bacteria (15°C and 37°C), anaerobic bacteria (37°C) and anaerobic sporeformers were analyzed statistically. Anaerobic sporeformers were estimated by the most probable number method. No significant differences ( $P < 0.01$ ) were found between rapid or conventional processing techniques in total aerobes and anaerobic sporeformers in fresh or cured muscle tissue. Total anaerobes were significantly greater ( $P < 0.05$ ) in conventional processing when compared to rapid processing. Results reveal no significant bacteriological problems in the rapid processing of pork prior to initial chilling.

**EFFECT OF NITRITE AND STORAGE TEMPERATURE ON THE ORGANOLEPTIC QUALITY AND TOXINOGENESIS BY *Clostridium botulinum* IN VACUUM-PACKAGED SIDE BACON.** D.L. COLLINS-THOMPSON, P.C. CHANG, C.M. DAVIDSON, E. LARMOND & H. PIVNICK. *J. Food Sci.* 39, 607–609 (1974)—The effect of nitrite and storage temperature and toxinogenesis by *Clostridium botulinum* in vacuum-packed side bacon was investigated. In two series of experiments (A & B) bacon packs were prepared with levels of 0, 50, 100, 150 and 200 ppm nitrite and inoculated with *C. botulinum* at  $10^2$  spores/g and  $10^4$  spores/g. Packs A were incubated at 20 and 30°C and packs B at 30°C only. Both were held for a maximum of 32 days and analyzed for toxin at intervals of 2, 4, 8, 16 and 32 days. At 20°C, none of the controls without nitrite was found to be toxic after 32 days. At 30°C, inhibition of toxin formation at the higher nitrite levels was observed at 32 days. Organoleptic evaluation of the bacon packs stored at 30°C showed about one-third of the toxic samples examined were acceptable to the panel.

**QUALITY ATTRIBUTES OF GROUND BEEF ON THE RETAIL MARKET.** P.A. KENDALL, D.L. HARRISON & A.D. DAYTON. *J. Food Sci.* 39, 610–614 (1974)—Each of three ground beef products, varying in price (based on lipid content) were purchased from each of three retail stores. Patties of each product were modified broiled at 177°C for 35 min. Products containing 10–20% lipid had less cooking loss than those containing 25–30% lipid, but lower-lipid, higher-priced beef cost more per 100g of cooked meat than higher-lipid, lower-priced beef. Percentage ether extract decreased after broiling raw ground beef containing 20–30% lipid, and increased slightly after broiling raw ground beef containing less than 12% lipid. Over-all acceptability was similar for all three products, but the leanest, highest-priced product rated less juicy, more mealy and lower in flavor than higher-lipid, lower-priced products.

**FREEZING OF COOKED MEAT: INFLUENCE OF FREEZING RATE AND RECONSTITUTION METHOD ON QUALITY AND YIELD.** B. JAKOBSSON & N. BENGTTSSON. *J. Food Sci.* 39, 615–619 (1974)—The combined effects of freezing rate, frozen storage and reconstitution method on meat quality and yield were studied in multifactorial experiments using pan-fried 1.5 cm slices of longissimus muscles and deep-fat fried meat patties. On the whole, a slight improvement in sensory quality and yield was seen with increasing freezing rate, except that liquid nitrogen immersion freezing influenced flavor negatively. In contrast with earlier results of ours with raw beef, an advantage was now seen for reconstitution after previous thawing over reheating directly from the frozen state, and pan frying gave better sensory quality but lower yield than continuous microwave reheating. Tenderness of sliced,

cooked beef tended to increase during frozen storage, while the opposite was earlier observed for frozen raw beef. Otherwise, a general trend was seen towards decreasing yield and sensory quality during frozen storage. A number of significant interactions between variables was obtained, suggesting that their influence on quality and yield is interrelated in a rather complex manner.

**CONTINUOUS PRODUCTION OF BLUE-TYPE CHEESE FLAVOR BY SUBMERGED FERMENTATION OF *Penicillium roqueforti*.** B.K. DWIVEDI & J.E. KINSELLA. *J. Food Sci.* 39, 620–622 (1974)—A two-stage continuous process for the production of Blue cheese flavor from lipolyzed milk fat by submerged fermentation of *Penicillium roqueforti* was developed. In the first stage, *P. roqueforti* was grown aerobically on a corn-steep liquor/sucrose medium at 25°C, pH 7, aeration rate 1 liter air/min/L medium, and dilution rate  $0.05 \text{ hr}^{-1}$ . The effluent air and culture over-flow were led to a maturing tank and allowed to age 24 hr. The mycelial culture was then pumped into a second fermentor to which lipolyzed milk fat was also fed continuously. An average 24 hr contact time between mold mycelium and fatty acids was maintained. The fatty acids and perhaps other components of the lipolyzed cream were metabolized by the *P. roqueforti* mycelium to produce a fluid product with a Blue cheese-type flavor. Small portions of this product were acceptably added to snack and party dips and salad dressings, as a Blue cheese replacement.

**A NUMERICAL METHOD OF SIMULATING THE AXISYMMETRICAL FREEZING OF FOOD SYSTEMS.** C. JOSHI & L.C. TAO. *J. Food Sci.* 39, 623–626 (1974)—A numerical method is developed to simulate the temperature progression of freezing a food system. It is assumed to have a constant density and to be macroscopically homogeneous during the freezing operation. The simulated results are compared with the measured temperature progression in the center of a beef slab immersed in a  $-39^\circ\text{F}$  bath.

**ANALYTICAL PREDICTION OF DRYING PERFORMANCE IN NON-CONVENTIONAL SHAPES.** E. ROTSTEIN, P.A. LAURA & M.E. de CEMBORAIN. *J. Food Sci.* 39, 627–631 (1974)—A study is made to develop a tool to predict drying performance of nonconventional shapes. Many food drying processes are diffusion-controlled. An approximated analytical solution to Fick's diffusion equation is given for bodies which have a cardioidal, corrugated, circular, epitrochoidal, square, or hexagonal cross section. A conformal mapping approach is used to obtain the concentration distribution and the result is integrated over the volume to yield analytical weight loss equations for each shape. The result is applied to compare drying performance of apples on an equal weight-equal cross sectional area or an equal weight-equal thickness basis.

**PREDICTION OF DIELECTRIC PROPERTIES IN OIL-WATER AND ALCOHOL-WATER MIXTURES AT 3,000 MHz, 25°C BASED ON PURE COMPONENT PROPERTIES.** R.E. MUDGETT, D.I.C. WANG & S.A. GOLDBLITH. *J. Food Sci.* 39, 632–635 (1974)—Measurements of oil-water emulsions show that dielectric properties are predicted at 3,000 MHz, 25°C by the noninteractive Fricke model for complex conductivity of colloidal suspensions. Similar measurements for methanol-water and ethanol-water show that dielectric properties are predicted at 3,000 MHz, 25°C by an empirical combination, designated as the interactive Fricke model, of the noninteractive Fricke model and the Debye models for pure polar liquids with single relaxation times. A tentative physical-chemical basis for prediction of dielectric properties in liquid food systems is suggested, which involves concepts of relative dielectric activities of major chemical constituents, extent of solvation and colloidal content and is based on mechanisms of dielectric behavior observed in aqueous mixtures at microwave frequencies.

# ABSTRACTS:

## IN THIS ISSUE

**PHENOLIC COMPOUNDS FROM THE BERRIES OF MOUNTAIN ASH, *Sorbus aucuparia*.** H. PYYSALO & T. KUUSI. *J. Food Sci.* 39, 636–638 (1974)—The polyphenolic extract from berries of Mountain Ash was separated into fractions in order to determine whether phenolic compounds are responsible for the bitter taste of the berries. The 18 phenols isolated included hydroxycinnamic acids, anthocyanins, leucoanthocyanins and flavanols, none of which had a strong bitter taste.

**PROTECTIVE EFFECT OF FORTIFIED SKIM MILK AS SUSPENDING MEDIUM FOR FREEZE DRYING OF DIFFERENT LACTIC ACID BACTERIA.** R.N. SINHA, A.T. DUDANI & B. RANGANATHAN. *J. Food Sci.* 39, 641–642 (1974)—The efficacy of fortified skim milk was tested for improving cell survival of 23 different cultures of freeze-dried lactic acid bacteria. Except in the case of *Streptococcus cremoris*, *Streptococcus thermophilus* (two strains), *Lactobacillus bulgaricus* and *Lactobacillus lactis*, all other freeze-dried cultures of lactic acid bacteria showed improved cell survival.

**COLLAGEN CHARACTERISTICS OF PRONGHORN ANTELOPE AND SHEEP MUSCLE.** W.G. KRUGGEL & R.A. FIELD. *J. Food Sci.* 39, 639–640 (1974)—Guanidine hydrochloride soluble intramuscular collagen (GSIC) obtained from purified longissimus intramuscular collagen of five yearling and five older doe antelope and from two lambs and two ewes varied with age but did not differ significantly ( $P < 0.05$ ) between species. Gel-disc electrophoresis of denatured GSIC showed a lower percentage of  $\alpha$ -chain and a higher percentage of  $\beta$ -component for antelope than for sheep. Total collagen content was lower in antelope than in sheep muscle. Therefore, the extreme tenderness in antelope muscle may result from a lack of intramuscular collagen rather than variation in collagen crosslinking.

**LONG-TERM STORAGE OF CELLS OF *Candida utilis*: A NOTE ON THEIR NUTRITIONAL EVALUATION.** J.S. ARAUJO NETO, K. MADI & A.D. PANEK. *J. Food Sci.* 39, 643–644 (1974)—Cells of *Candida utilis* when stored for some years under uncontrolled conditions develop undesirable properties which impair the previously normal biological value of their proteins. In spite of the simultaneous addition of casein (1:1 on a crude protein basis) and of 0.2% DL-methionine, the long-stored cells did not support reasonable growth rates. The chemical and histo-pathological data are quite normal and intestinal disturbances were the only noticed ill-effects.

### Erratum Notice

- *J. Food Sci.* 39(2): 288–292 (1974), V. Yeo, G.H. Wellington and K.H. Steinkraus: “Effects of soy curd on the acceptability and characteristics of beef patties.” On page 288, Experimental, line 20 and following, change to: “While the soy milk was still hot (above 75°C), 0.4% of CaSO<sub>4</sub> based on the soy milk weight was stirred in.”

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SIMON C. M. KWOK<sup>1</sup>, HARVEY T. CHAN JR.<sup>1</sup>, T. O. M. NAKAYAMA<sup>2</sup>  
and JOHN E. BREKKE<sup>1</sup>  
USDA and University of Hawaii, Honolulu, Hawaii

## PASSION FRUIT STARCH AND EFFECT ON JUICE VISCOSITY

### INTRODUCTION

PASSION FRUIT, indigenous to the American tropics, is now grown in most of the tropical countries of the world. In Hawaii the yellow passion fruit, *Passiflora edulis* f. *flavicarpa* Degener, is the basis of the entire passion fruit juice industry while in Australia, Ceylon, and India, the purple passion fruit, *P. edulis* f. *edulis*, is more important (Martin and Nakasone, 1970).

Chan et al. (1972) reported that juice of the yellow variety differs from the purple in total acid content and in the relative proportions of each of the acids. Flavor differences between yellow and purple passion fruit juices have been reported by Parliment (1972) and by Hiu and Scheuer (1961). Wenkam and Miller (1965) reported that the yellow variety had a much higher vitamin A value than the purple.

Pruthi (1963) reported the starch content of purple passion fruit juice as 1.0–3.7%; no value was reported for the yellow variety. Cillie and Joubert (1950) isolated and characterized the starch from the purple variety. They determined that the starch granules gelatinized at 30–35°C. They also reported that passion fruit starch was almost pure amylopectin, containing only 1.2% amylose. References to the effect of processing on passion fruit starch have been made by Seale and Sherman (1960) and Mollenhauer (1954). Seale and Sherman (1960) noted that the high starch content of passion fruit juice caused gelatinous deposits to accumulate on the heating surfaces of the heat exchanger which resulted in localized scorching with a resultant drop

in heat-exchanger efficiency and deterioration in juice flavor. Mollenhauer (1954) noted that when purple passion fruit juice was heated, viscosity increased rapidly between 50–81°C. He attributed these changes to the gelatinization of starch.

Preliminary work at this laboratory on the concentration of tropical fruit juices indicated that viscosity is an important factor in determining the degree of concentration which can be attained. Because starch may contribute to the viscosity of passion fruit juice, we investigated the starch of purple and yellow passion fruit. Reported herein are the results of this study.

### EXPERIMENTAL

#### Passion fruit juice

Yellow and purple passion fruit were harvested from the Hawaii Agricultural Experiment Station Farm. The juice was expressed from the pulp through four layers of cheesecloth and stored at –17.8°C.

#### Isolation of starch

Starches were isolated from the juices of purple and yellow passion fruit by repeated centrifugation and washing by Cillie and Joubert's (1950) method.

#### Characterization of isolated starch

The amylose content of the isolated starch was estimated by the standard potentiometric

Table 1—Properties of isolated passion fruit starch

	<i>P. flavicarpa</i>	<i>P. edulis</i>
% Iodine affinity	1.8	1.2
% Amylose <sup>a</sup>	8.7	5.8
Average granule size, $\mu$ m	7.8	6.4
Gelatinization temp. range, °C	58.5–67.0	58.5–66.5
$\lambda$ Max of starch-iodine complex, nm	580	570

<sup>a</sup> Calculated from  $\frac{\text{Iodine affinity of starch}}{\text{Iodine affinity of amylose}} \times 100$

Table 2—Properties of juices of yellow, *P. flavicarpa*, and purple passion fruit, *P. edulis*

	<i>P. edulis</i>	<i>P. flavicarpa</i>	<i>P. flavicarpa</i> Starch enriched	<i>P. flavicarpa</i> pH adjusted <sup>a</sup> starch enriched
pH	4.2	2.8	2.8	4.2
Starch content %	0.74	0.06	0.74	0.74
Relative viscosity <sup>b</sup> of unheated juice	2.1	2.5	2.5	2.4
Relative viscosity <sup>b</sup> of heated juice	4.0	2.7	3.4	4.1

<sup>1</sup>Hawaii Fruit Laboratory, USDA, ARS, Honolulu, HI 96822

<sup>2</sup>Dept. of Food Science & Technology, University of Hawaii, Honolulu, HI 96822

<sup>a</sup> pH adjusted with 10N KOH

<sup>b</sup> Ratio of sample flow time to flow time of water

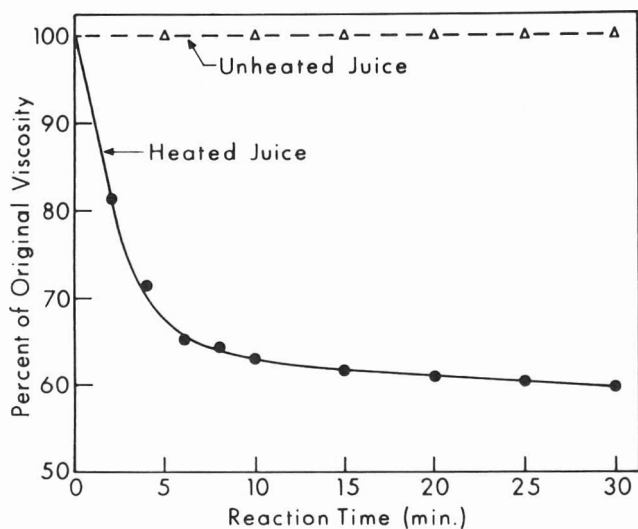


Fig. 1—Effect of  $\alpha$ -amylase on purple passion fruit juice, pH 4.2.

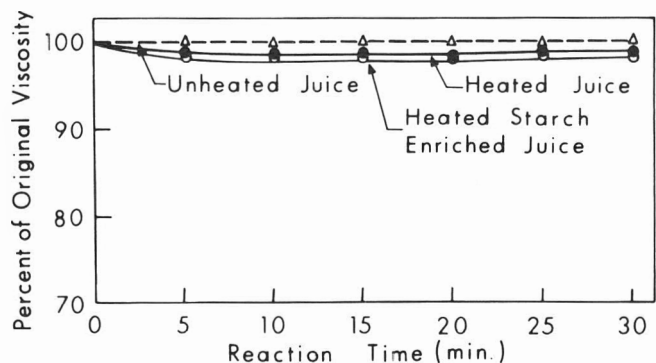


Fig. 2—Effect of  $\alpha$ -amylase on yellow passion fruit juice, pH 2.8.

method described by Schoch (1964). The isolated passion fruit starch was defatted by refluxing 2 hr in 85% methanol, filtered in vacuo, and dried overnight at 50°C. A Beckman PHASAR-I digital pH meter with platinum electrode was used for the titration. The titration was performed on three or more replications.

The size of starch granules was determined microscopically with a micrometer eyepiece calibrated with a Petroff-Hauser hemacytometer. Averages of 21 or more observations on three different samples of each variety were taken. The gelatinization of starch granules was determined by placing an aqueous suspension of starch (3 mg/ml) in water baths of various temperatures, then observing the loss of birefringence with a polarizing microscope. The temperatures of gelatinization ranges were taken as the range between first evidences of loss of birefringence and final, total loss. The gelation temperature determinations were done in triplicate.

#### Determination of starch content

The rapid colorimetric method of Carter and Neubert (1954), with slight modifications, was used to determine the starch content of the passion fruit juices. 5g of passion fruit juice were mixed with 20 ml of 7.8N perchloric acid for 5 min. The mixture was then diluted with distilled water (100 ml for yellow passion fruit and 500 ml for purple), and filtered through glass wool. To a 10-ml aliquot, 0.2 ml of a potassium iodide-iodine solution (2 mg of iodine and 20 mg of potassium iodide/ml) was added. Absorbance was measured at the wavelengths corresponding to the maximum absorption (580 nm and 570 nm for the yellow and purple passion fruits, respectively), using a Perkin-Elmer 202 spectrophotometer. An iodine-iodide solution of equal concentration was used as reference. The percentages of starch in the juices were determined from the standard curves which were prepared from known amounts of isolated starch from each of the two passion fruit varieties. Starch determinations were performed in triplicate.

#### Effect of heating on viscosity of passion fruit juice

About 100 ml of passion fruit juice were placed in a 250-ml volumetric flask. The flask was closed with a rubber stopper through which a thermometer was inserted. The juice then was heated to 70°C with stirring and cooled immediately in ice. Unheated juice was used as control. Flow times of the heated and unheated juices were measured on six replications in an Ostwald viscometer (Cannon-Fenske Viscometer Tube, size no. 300) with 10 ml juice at 30°C. Relative viscosities then were calculated from the ratio of flow time of sample to that of distilled water.

#### Effect of $\alpha$ -amylase on viscosity of passion fruit juice

The effect of  $\alpha$ -amylase on viscosity was

studied in heated and unheated juice. Fungal  $\alpha$ -amylase (Miles Laboratories, Inc.), 10 mg, was mixed with 10 ml of passion fruit juice at 30°C. The reaction mixture then was placed in an Ostwald viscometer and incubated at 30°C. Flow times were taken at 2–5 min intervals. Results were calculated from the average of six replications.

## RESULTS & DISCUSSION

THE AMYLOSE CONTENTS of isolated passion fruit starch were 8.7% and 5.8% for the yellow and purple passion fruits, respectively (Table 1). These values were much higher than that of 1.2% amylose for purple passion fruit reported by Cillie and Joubert (1950).

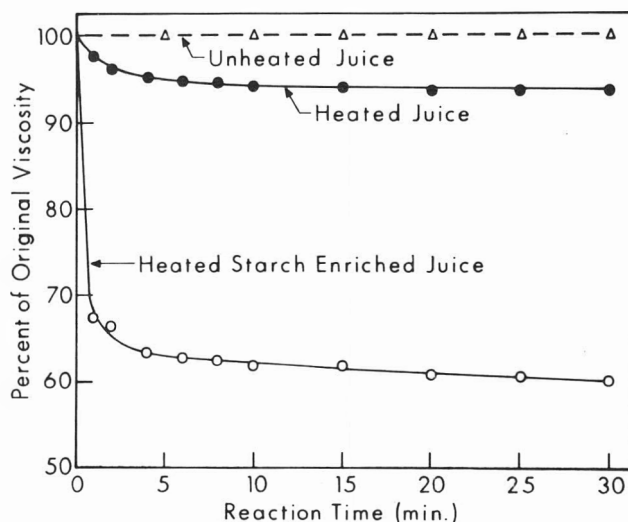


Fig. 3—Effect of  $\alpha$ -amylase on yellow passion fruit juice, pH adjusted to 4.2.

Microscopic examination showed that morphologically the starch granules isolated from the two varieties of passion fruits were the same. For both varieties, the range of granule size was 4.0–12.2  $\mu\text{m}$ . However, the mean granule diameter for purple passion fruit was 6.4  $\mu\text{m}$  ( $\sigma = 1.91$ ) and for yellow 7.8  $\mu\text{m}$  ( $\sigma = 3.9$ ). The ranges of starch gelatinization temperatures of purple, 58.5–67.0, and yellow, 58.5–66.5, were nearly identical and corresponded to the range at which a rapid increase in viscosity of purple passion fruit juice was noted by Mollenhauer (1954). However, the gelation temperatures, 58.5–67.0°C, reported here are much higher than the 30–35°C reported by Cillie and Joubert (1950) for purple passion fruit.

The effects of heating yellow and purple passion fruit juices beyond the gelation point of their starches are shown in Table 2. When juices were heated to 70°C, relative viscosity of purple fruit juice almost doubled but that of yellow fruit juice increased only slightly. The differences in the effect of heat on the viscosities of yellow and purple passion fruit are due to their differences in starch content. This fact was verified in further experiments in which the juice of yellow passion fruit was enriched, with starch isolated from yellow passion fruit, to the level of purple passion fruit, 0.74%. The results indicate that the viscosity of starch-enriched yellow passion fruit juice is nearly the same as that of purple passion fruit juice when heated beyond the gelation point of their starches. When the pH of the starch-enriched yellow passion

fruit juice was increased to equal the pH of purple passion fruit juice, the viscosity of yellow fruit juice equaled that of purple fruit juice.

Figures 1 and 2 show the action of  $\alpha$ -amylase on heated and unheated purple and yellow passion fruit juice. The results indicated that fungal  $\alpha$ -amylase rapidly reduced the viscosity of heated purple passion fruit juice but had no effect on unheated juice. The lack of  $\alpha$ -amylase effect on unheated passion fruit juice may be further explained by work reported by Walker and Hope (1963) who showed that fungal  $\alpha$ -amylase will not degrade intact maize starch granules but will attack granules that have been gelatinized.

Fungal  $\alpha$ -amylase did not reduce the viscosity of either heated or unheated yellow passion fruit juice at its natural pH of 2.8 (Fig. 2). Enrichment of the juice with starch did not enhance the action of  $\alpha$ -amylase (Fig. 2). When the pH of yellow passion fruit juice was adjusted to that of the purple variety (pH 4.2),  $\alpha$ -amylase action was observed in the starch-enriched and in the unenriched samples (Fig. 3). Apparently, the pH of yellow passion fruit juice is beyond the effective pH range of  $\alpha$ -amylase.

The results of this study show that the properties of the starches from juices of yellow and purple passion fruit are quite similar. Differences in the viscosities of juices of the two varieties in their responses to heat and  $\alpha$ -amylase are due principally to their differences in starch concentration and in pH. The results of this study also show that  $\alpha$ -amylase could

be used to reduce the viscosity of the juice of purple passion fruit, after the starches have been gelatinized.

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## DETERMINATION OF VOLATILES BY VAPOR HEADSPACE ANALYSIS IN A MULTI-PHASE SYSTEM: d-LIMONENE IN ORANGE JUICE

### INTRODUCTION

VAPOR HEADSPACE analysis by gas-liquid chromatography is commonly used in the food field as a means of identifying and determining relative concentration of volatile compounds in foods and model solutions (Wolford et al., 1963; Teranishi et al., 1963, 1966; Schultz et al., 1964, 1967; Chandrasekaran and King, 1971; etc.). The technique consists of equilibrating the vapor in contact with the liquid phase where the volatiles are present, at a fixed temperature in a closed vessel. An aliquot of the vapor is removed and injected in a chromatograph, now usually equipped with a flame-ionization detector. The area obtained for a given peak is assumed to be directly proportional to the liquid concentration of that substance. Proper calibration with a solution of known concentration further allows determination of absolute concentrations (Buttery et al., 1969; Massaldi and King, 1973).

A headspace analysis technique has also been developed (Massaldi and King, 1973) to determine solubilities of scarcely soluble volatile compounds in water and aqueous solutions. When one is dealing with very insoluble substances or with substances of moderate solubility which are present at relatively high concentration levels, the possibility exists that the solubility limit is exceeded in the liquid phase. In this case, the vapor concentration is independent of the liquid concentration, and the peak area in a vapor sample is no longer sensitive to the concentration in the liquid. The aroma components in a liquid food are often substances of low water solubility, although for most of these compounds the natural concentration is much lower than the solubility. However, important exceptions can be pointed out. In the case of coffee, recovered aroma species can be dissolved in coffee oil and added back to coffee extract to enhance the flavor, the result being a distribution of the aroma components between the aqueous and oil phases. In orange juice d-limonene, which

accounts for 90–95% of orange oil, is known to be part of the water-insoluble fraction of the aroma. Further, it has been suggested (Scott et al., 1969; Mizrahi and Berk, 1970) that orange oil combines somehow with the lipid fraction of the "cloud" in orange juice. It was found in the present work that d-limonene actually dissolves in a third phase, very probably the lipid fraction, in a way consistent with these observations. Under the conditions in orange juice, not only does the essence of oil separate out as another liquid phase, but also this liquid phase is in equilibrium with the oil dissolved in both the aqueous and lipid phases.

Scott and Veldhuis (1966) developed a chemical method to estimate recoverable oil in orange juice, reported as d-limonene. The method is based on extraction and distillation of the oil, with further titration of d-limonene. If all the oil is extracted from the juice, the procedure yields a direct estimation of the total oil content, characterized as d-limonene. In the present paper, an alternative technique, based on vapor headspace analysis, is presented for the determination of d-limonene in synthetic emulsions and in orange juice. The method is general, however, in the sense that it allows determination of any volatile components distributed between phases. It is also felt that it can provide a better understanding of the problem of phase equilibria in a liquid food.

### BASIS FOR THE METHOD

When  $N_{Si}$  moles of a solute are allowed to partition between condensed phases L and F in equilibrium with a vapor phase V, the following mass balance holds,

$$N_{Si} = yN_V + x_L N_L + x_F N_F \quad (1)$$

where  $N_V$ ,  $N_L$  and  $N_F$  are the total number of moles in phases V, L and F respectively, and  $y$ ,  $x_L$  and  $x_F$  are the mole fractions of the substance in those phases. In Equation (1), it is assumed that a pure solute phase is not present, e.g., that the equilibrium partial pressure of the solute is sufficiently less than its vapor pressure.

By defining an equilibrium ratio  $K$  as,

$$K = \frac{y}{x_L} \quad (2)$$

and a distribution coefficient  $K_F$  as,

$$K_F = \frac{x_F}{x_L} \quad (3)$$

and substituting Equations (2) and (3) in Equation (1), one obtains,

$$N_{Si} = yN_V + \frac{y}{K}N_L + \frac{y}{K}K_F N_F \quad (4)$$

which, solved for the vapor mole fraction, yields,

$$y = \frac{N_{Si}}{N_V + N_L/K + K_F N_F/K} \quad (5)$$

When phase F is not present, Equation (5) reduces to,

$$y = \frac{N_{Si}}{N_V + N_L/K} \quad (6)$$

### Synthetic emulsions of d-limonene in aqueous solutions

In this case, two liquid phases, a dispersed, pure d-limonene phase and a continuous, aqueous phase are present. If an aliquot of this emulsion is mixed with a volume of water sufficient to dissolve all the d-limonene, and the new, diluted solution is equilibrated with a vapor phase in a closed flask, then Equation (6) holds, where subscript L stands for the aqueous phase. Under these conditions, for fixed values of  $N_V$  and  $N_L$  and assuming that  $K$  does not change with d-limonene concentration,  $y$  is directly proportional to  $N_{Si}$ , where  $N_{Si}$  is the number of moles of d-limonene originally present in the aliquot. If the dilution with water creates a final solute content below 5%, the value of  $K$  in Equation (6) can be taken as that for d-limonene in pure water. At 25°C,  $K = 1.392 \times 10^3$  in pure water (Massaldi and King, 1973).  $K$  is obtained as  $K = \gamma P_V^0/P$ , where  $\gamma$  = activity coefficient of d-limonene,  $P_V^0$  = vapor pressure of d-limonene and  $P$  = total pressure.  $\gamma$  is reported by Massaldi and King (1973) to be constant for  $yP/P_V^0 \leq 0.8$ .

In order for the d-limonene content of the original emulsion to be determined,  $N_{Si}$  must be calculated. A calibration may be made by headspace analysis of a similar flask with the same  $N_V$  and  $N_L$  and with a known amount of d-limonene,  $N_{SR}$ , added. Equation (6) applied to the

<sup>1</sup>Present address: Departamento de Tecnología Química, Facultad de Ciencias Exactas, UNLP, 47 y 115, La Plata, República Argentina



unknown flask and to the calibration flask gives,

$$\frac{y}{y_R} = \frac{A}{A_R} = \frac{N_{Si}}{N_{SR}} \quad (7)$$

where A is the peak area on the chromatograph and subscript R stands for the reference (calibration) flask. The concentration in the original sample can now be calculated in any desired units as,

$$C = \frac{N_{Si}}{w} = \frac{A}{A_R} \cdot \frac{N_{SR}}{w} \quad (8)$$

where w is the quantity of the aliquot of emulsion initially added to the water.

#### d-Limonene in orange juice

In natural-strength or concentrated citrus juices, d-limonene is distributed between three liquid phases: the aqueous, the lipid and the oil phases. By carrying out the same dilution procedure with an aliquot w in water, as was described for the synthetic emulsions, the oil phase can be made to disappear, but now Equation (5) should be used, since the partition coefficient  $K_F$  is high enough to allow a substantial amount of d-limonene still to be dissolved in the lipid phase F. For this reason, if a direct headspace analysis and calibration is used as in the case for synthetic emulsions, the following relationship is obtained from Equations (5) and (6),

$$\begin{aligned} \frac{y}{y_R} &= \frac{A}{A_R} \\ &= \frac{N_{Si}}{N_{SR}} \cdot \frac{N_v + N_L/K}{N_v + N_L/K + K_F N_F/K} \end{aligned} \quad (9)$$

and the concentration is then given by,

$$\begin{aligned} C &= \frac{N_{Si}}{w} \\ &= \frac{A}{A_R} \cdot \frac{N_{SR}}{w} \left( 1 + \frac{K_F N_F}{K N_v + N_L} \right) \end{aligned} \quad (10)$$

where w is again the amount of initial sample. Equation (10) shows that in this case, even for known values of  $K N_v$  and  $N_L$ , another variable,  $K_F N_F$ , must be determined independently in order to calculate the total concentration in the juice.

#### The lipid extraction parameter

The concentration and composition of lipid constituents in citrus juices depend upon the nature of the fruit, the extraction procedure, the amount of pulp present and probably processing variables for commercial juices. For this reason, any property associated with the lipid fraction should be determined for the particular juice under study, in relation to the juice strength. In the present case we

are interested in the d-limonene absorptive capacity of the lipid fraction at the dilution conditions, represented by  $K_F N_F$ . It is convenient to define a new parameter, which should be uniquely characteristic of the juice under the conditions of the analysis, in the form,

$$\lambda_F = \frac{K_F N_F}{wb} \quad (11)$$

where w is the aliquot quantity (grams) of juice and b is the fraction dissolved solids in the juice.  $\lambda_F$  represents the d-limonene absorptive capacity of the lipid fraction per unit weight and per unit dissolved solids content of the juice.

$\lambda_F$  for a juice, when added to the dilution flask, can be determined by making use of Equation (5) as follows: Substituting Equation (11) into Equation (5) gives,

$$y = \frac{N_{Si}}{N_v + N_L/K + wb\lambda_F/K} \quad (12)$$

If  $N_s$  additional moles of d-limonene are added to a similar dilution flask, where the same aliquot of the same juice is dissolved in a volume of water such that saturation is not reached after equilibrium, Equation (12) becomes,

$$y = \frac{N_s + N_{Si}}{N_v + N_L/K + wb\lambda_F/K} \quad (13)$$

By using increasing values of  $N_s$ , with  $N_v$ ,  $N_L/K$  and  $N_F$  constant, a straight-line relationship between y and  $N_s$  is obtained, with slope,

$$\frac{dy}{dN_s} = \frac{1}{N_v + N_L/K + wb\lambda_F/K} = a \quad (14)$$

and intercept,

$$N_s \Big|_{y=0} = -N_{Si} \quad (15)$$

If  $N_s$  is the number of moles corresponding to the volume s of d-limonene added to each flask and  $A_0$  is the peak area for a saturated solution of d-limonene in water, the slope a can be calculated as,

$$\begin{aligned} a &= \frac{dy}{dN_s} = \frac{y^*}{\rho_{d-1}} \frac{d(y/y^*)}{ds} \\ &= \frac{y^*}{\rho_{d-1}} \frac{d(A/A_0)}{ds} \end{aligned} \quad (16)$$

where  $\rho_{d-1}$  is the molar density of d-limonene ( $6.17 \times 10^{-3}$  gmole/ml at 25°C).  $y^*$  is the d-limonene vapor mole fraction in equilibrium with pure, liquid d-limonene. At 25°C,  $y^* = 1.9 \times 10^{-3}$  (Massaldi and King, 1973).

Rearranging Equation (14) gives,

$$\lambda_F = \frac{1}{wb} \left( \frac{K}{a} - K N_v - N_L \right) \quad (17)$$

The values of w, b,  $N_v$  and  $N_L$  are known from the experimental conditions. Again, if the final solids content is below 5%, K for d-limonene in water can be used.

Once  $\lambda_F$  is known from Equations (11) and (17), the concentration in the juice can be calculated from Equation (10) in the form,

$$C = \frac{A}{A_R} \frac{N_{SR}}{w} \left( 1 + \frac{wb\lambda_F}{K N_v + N_L} \right) \quad (18)$$

Alternatively,  $C = N_{Si}/w$  may be obtained from the intercept through Equation (15), if enough points are obtained for the straight line represented by Equation (13) to be drawn.

## EXPERIMENTAL

### Preparation of samples

**Emulsions.** Synthetic emulsions of d-limonene in 25% and 10% sucrose solution were prepared by adding an excess of the solute to sucrose solutions pre-chilled to 0°C, and then shaking vigorously for 1 min. Emulsions prepared in this way were found to be stable for more than 24 hr when kept at 0°C in a refrigerator.

**Orange juice.** Specimens of orange juice from a number of sources were tested, including fresh squeezed (FS), commercial pasteurized, single-strength (CP) and reconstituted commercial frozen concentrate (CFC). The FS juice was obtained from fresh Navel oranges which were purchased locally. The juice was extracted with a standard home reamer and was filtered through a 1.2 mm mesh to remove the coarse pulp. The juice was pasteurized by stirring at 95°C for 1 min and was then cooled by immersion of the flask in a methanol bath at -30°C. CP juice (produced in Florida and denoted as Brand A) and CFC juice (produced in Florida and denoted as Brand E) were purchased locally. Before use, the CFC juice was partially reconstituted to strengths of 25° and 10° Brix, as measured by an Abbe refractometer, using the calibration for sucrose solutions as the standard. The oil level was either the natural one (FS and CP juices) or that remaining after reconstitution (CFC juice). Stocks of the samples were kept in the refrigerator no more than 6 hr before use.

In one experiment, 25% CFC juice was centrifuged at  $13,200 \times G$  for 30 min in a Sorvall, Model RC-2 Centrifuge. Two fractions were obtained, 9.1% weight of pulp sediment and 90.9% weight of an opaque upper phase, containing colloiddally suspended matter (cloud) and labelled Serum 1.

### Dilution procedure and analytical technique

Several 250-ml flasks were chosen to have the same volumetric capacity up to the top, within 5%. Constant liquid and vapor volumes were left in the flasks after dilution of the juice and emulsion samples with water (L = 160 ml, and V = 100 ml, in all cases). Under these conditions, dissolution of all the d-limonene was ensured for a range of d-limonene concentrations of 10–500 mg/liter in the initial sample, and for weights of sample between 6 and 12g. The solubility of d-limonene at 25°C in water and sugar solutions is of the order of 10 ppm (Massaldi and King, 1973). A stirred thermo-

static bath was used to equilibrate the flasks at  $25 \pm 0.1^\circ\text{C}$  before the analysis. Vapor samples were analyzed with a Varian Aerograph 1400 Series, flame ionization chromatograph, under the same column and operating conditions as described elsewhere (Massaldi and King, 1973).

**Determination of  $\lambda_F$**

For the various juices a constant amount of sample, 7 or 11g, was diluted in the 250-ml flasks. Increasing microquantities of d-limonene were added to each flask in the same manner as described by Massaldi and King (1973), with careful attention to ensuring that a known and controlled volume of d-limonene was delivered. Equilibration was allowed, and headspace analysis was performed on each flask. Simultaneously, a reference peak area was obtained for a sample of vapor taken from above a saturated solution of d-limonene in water, held at the same temperature.

**RESULTS & DISCUSSION**

**Synthetic d-limonene emulsions**

A number of specimens were made up from sugar solutions to which known amounts of d-limonene, ranging from 1–12 times the solubility limit, were added. Analyses of these through the dilution technique, using Equation (8) to compare with a reference solution, gave

good and reproducible results, generally agreeing within 2%.

**Orange juice**

Figure 1 shows the normalized peak area found for various values of  $s$ , the volume of pure liquid d-limonene added, for 7-g samples of the two juices indicated. Figure 2 shows similar results obtained for the addition of pure, liquid d-limonene to pure water (Massaldi and King, 1973). If the extractive, lipid phase were not present in orange juice, the lines in Figure 1 would be expected to have the same slope as that of the line for pure water in Figure 2. Because of extraction into the lipid phase the slopes of the lines of Figure 1 are lower, in accordance with Equation (14).

Figures 3 and 4 show the normalized peak area as a function of the amount of pure, liquid d-limonene added for CFC orange juice and for Serum 1. Two different amounts of sample were used in each case, so as to test the consistency of the method. It is apparent in both cases that the line for the 11-g sample shows a lesser slope than that for the 7-g sample, as is predicted through the effect of  $w$  in

Equation (14). Similarly, the 11-g samples have more negative intercepts with the horizontal axis than do the 7-g samples, as is to be expected from Equation (15), since a greater quantity of d-limonene is present in the larger sample.

Table 1 shows values of the lipid extraction parameter  $\lambda_F$  calculated for the various juices using Equations (14) and (16). For CFC juice and Serum 1, the values of  $\lambda_F$  determined for the two different sample weights fall within 8% of one another. It is also interesting to observe that Serum 1 retains 67% of the lipid extraction capability of the CFC juice, despite the removal by centrifugation of suspended matter in the amount of 9.1% of the juice. This is in concordance with the earlier conclusion that the d-limonene-holding capacity of suspended matter is primarily associated with the colloidal fraction of the juice (cloud) (Scott et al., 1969; Mizrahi and Berk, 1970).

Also shown in Table 1 are d-limonene contents of the juice specimens at the stated dissolved-solids contents, determined from the intercepts of the lines (Equation (15)) multiplied by the ratio of

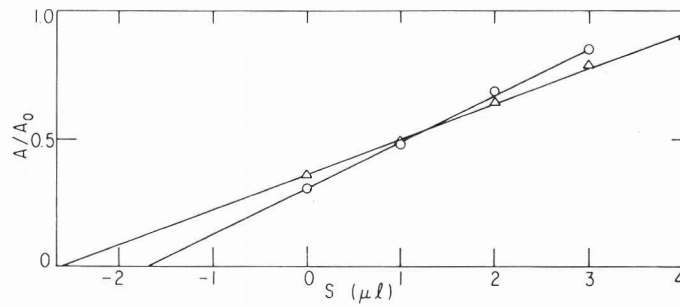
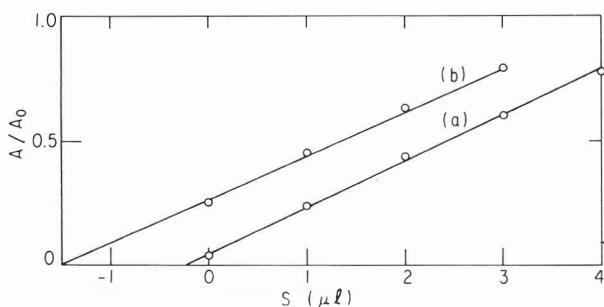


Fig. 1—Normalized Peak Area vs. Volume of d-Limonene Added for 7-Gram Samples of (a) FS Navel Orange Juice and (b) CP Commercial Pasteurized Orange Juice.

Fig. 3—Normalized Peak Area vs. Volume of d-Limonene Added for 7-Gram (○) and 11-Gram (△) Samples of CFC Frozen Orange Concentrate, Reconstituted to 25 wt. % Dissolved Solids.

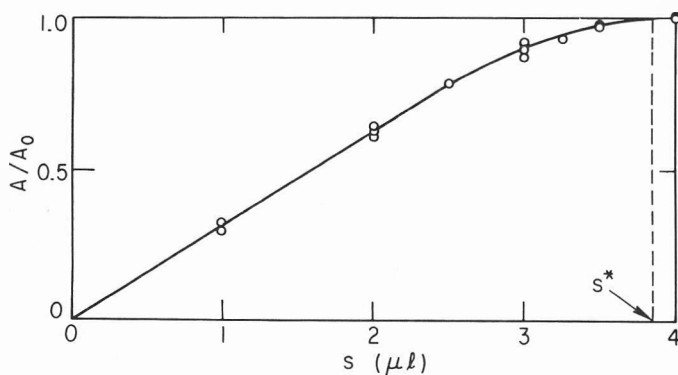


Fig. 2—Normalized Peak Area vs. Volume of d-Limonene Added to Pure Water (Massaldi and King, 1973).

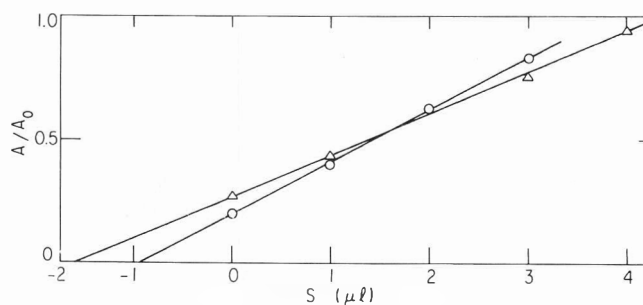


Fig. 4—Normalized Peak Area vs. Volume of d-Limonene Added for 7-Gram (○) and 11-Gram (△) Samples of Serum 1.

Table 1—Values of lipid parameter  $\lambda_F$  and d-limonene content determined for different orange juices

Type of juice <sup>a</sup>	Dissolved solids (wt %)	Sample weight Used (w) (g)	d-Limonene content (ppm, wt)	$\lambda_F$ (g mol/g)
FS	12.7	7	30	9.9
CP	11.2	7	180	13.9
CFC	25.0	7	210	5.9
CFC	25.0	11	210	6.3
Serum 1	25.0	7	110	3.9
Serum 1	25.0	11	120	4.2

<sup>a</sup> FS = fresh squeezed; CP = commercial pasteurized, single-strength; CFC = reconstituted commercial frozen conc.

Table 2—Comparison between concentrations calculated with and without allowing for parameter  $\lambda_F$  (CFC juice, 25% dissolved solids)

Weight (w) (g)	Peak area	Correct conc (mg/liter)	Erroneous conc (mg/liter)
10.4	7.54	210	99
8.8	7.10	215	111
8.2	6.90	218	115
5.3	5.50	223	142

the density of d-limonene (0.842 g/ml) to the sample weight. For CFC juice and Serum 1 the d-limonene contents found for the two different sample weights agree well.

Finally, Table 2 gives an indication of the degree of importance of allowing for the partitioning of d-limonene into the lipid phase. Peak areas are reported for d-limonene headspace analysis above samples of different weights of CFC juice, reconstituted to 25 wt % dissolved solids. The correct concentration of d-limonene calculated from Equation (18) is shown and may be seen to be constant within the scatter of data. Also shown are "erroneous concentrations," obtained from Equation (8), referenced to the peak area observed for d-limonene above pure water and ignoring the extractive capacity of the lipid phase. These latter values are much lower and show considerable trend with sample size. The percentage of the d-limonene contained in the various phases within the dilution flask can be computed from the ratios of the three terms in the denominator of Equation (14). Under conditions of the present experiments, between 32 and 56% of the d-limonene was located in the lipid phase, and of the remainder the amount in the aqueous phase was 1.56 times the amount in the vapor phase.

## NOTATIONS

- a =  $dy/dN_s$ , given by Equation (14)  
 A = Peak area (gas chromatograph)  
 A<sub>o</sub> = Peak area for vapor above saturated solution of d-limonene  
 A<sub>R</sub> = Peak area for vapor above reference solution  
 b = Weight fraction dissolved solids  
 C = Concentration of solute in sample (total, allowing for all phases)  
 K =  $y/x_L$  at equilibrium ( $1.392 \times 10^3$  for d-limonene in water at 25°C)  
 K<sub>F</sub> =  $x_F/x_L$  at equilibrium  
 N<sub>F</sub> = g moles of F phase (lipid)  
 N<sub>L</sub> = g moles of L phase (aqueous)  
 N<sub>s</sub> = g moles of additional solute (d-limonene) added  
 N<sub>si</sub> = g moles of solute (d-limonene) initially present  
 N<sub>sR</sub> = N<sub>s</sub> for reference solution  
 N<sub>v</sub> = g moles of vapor phase  
 P = Total pressure  
 P<sub>v</sub><sup>o</sup> = Vapor pressure of pure solute (d-limonene)  
 s = Volume of additional solute (d-limonene) added  
 w = Amount of sample added to dilution flask (g)

- $x_F$  = Mole fraction of solute in F phase (lipid)  
 $x_L$  = Mole fraction of solute in L phase (aqueous)  
 y = Mole fraction of solute in vapor phase  
 y<sub>R</sub> = y for reference solution  
 y\* = y in equilibrium with pure solute ( $1.9 \times 10^{-3}$  for d-limonene at 25°C)  
 Greek  
 $\gamma$  = Activity coefficient of solute in solution  
 $\lambda_F$  = Lipid extraction parameter, defined by Equation (11)  
 $\rho_{d-1}$  = Molar density of solute ( $6.17 \times 10^{-3}$  g/ml for d-limonene at 25°C)

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## VOLATILES RETENTION DURING FREEZE DRYING OF SYNTHETIC EMULSIONS

### INTRODUCTION

THE MAINTENANCE of flavor and aroma of foods during dehydration processes is known to be closely related to the degree of retention of various volatile organic compounds. Model solutions have been extensively used for controlled studies of the factors affecting the loss of volatile aroma substances. These model aqueous solutions typically contain saccharides, such as sucrose, fructose, glucose or higher molecular weight dextrans, along with low concentrations of simple volatile aromatics, such as alcohols, ketones and esters.

However, liquid foods consist of a more complex mixture of substances, in which very often more than one phase is present (e.g., dispersed solid matter or emulsions), thereby complicating the simpler, homogeneous system simulated by the model solutions. Concentrated aroma oils are often added to make up for losses in previous stages of processing. This procedure not only increases the relative proportion of heterogeneous constituents in the food, but also can further extract other, different aroma substances from the aqueous phase into the oil phase.

The purpose of the present work was to determine and interpret the retention behavior of volatile organic substances, at levels of concentration both below and above their limit of solubility, during freeze drying of sucrose solutions.

#### Aroma retention in freeze drying

**Homogeneous solutions.** For processing conditions which lead to significant retentions, the retention of volatile compounds during dehydration of homogeneous model solutions and liquid foods can be interpreted in terms of a simple diffusion mechanism (Thijssen and Rulkens, 1968; Menting et al., 1970). For quantitative prediction of retentions and concentration profiles of aroma compounds within the medium being dried, it is appropriate to work with a ternary diffusion model, considering water, dissolved solids and the volatile species as separate components (Chandrasekaran and King, 1972).

Figure 1 shows a schematic of the separation of a liquid food or model solution into ice crystals and interstitial unfrozen concentrate during freezing, followed by sublimation of the ice crystals during freeze drying. Diffusivities have not been measured for the conditions of solute concentration and temperature prevailing in the interstitial concentrate webs during freeze drying, but it is still possible to interpret volatiles retention data in freeze drying qualitatively by application of a simple diffusion model to the regions of interstitial concentrate between ice crystals, provided the volatiles are present in homogeneous solution within the concentrate. Thus, the volatiles retention should increase with decreasing values of the Fourier group  $Dt/L^2$ , where  $D$  is the diffusivity of the volatile solute within the concentrate,  $t$  is the time of exposure of the concentrate webs after the ice front passes and before they achieve sufficient dryness to prevent loss, and  $L$  is the thickness of interstitial webs of concentrate (Thijssen and

Rulkens, 1968; King, 1970, 1971). This simple analysis correctly explains (King, 1970, 1971) the experimentally observed beneficial effects upon volatiles retention caused by higher initial dissolved solids content, slower rates of freezing and smaller piece sizes (Sauvageot et al., 1969; Flink and Karel, 1970; Rulkens and Thijssen, 1972). "Collapse" at too high a frozen-zone temperature during freeze drying causes massive aroma loss because of flow, loss of structure and/or frothing of the interstitial concentrate (Bellows and King, 1973).

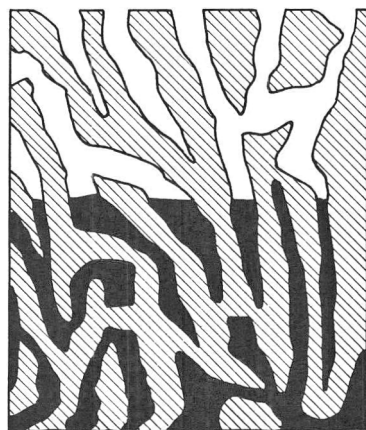
**Heterogeneous suspensions.** From physical considerations, one can expect the formation of a second liquid or dispersed solid phase to have a potentially substantial effect upon both the mechanism and magnitude of volatiles retention during freeze drying. Some preliminary reinforcement for this concern comes from the work of Sauvageot et al. (1969), where very different retentions of d-limonene, a compound which is probably emulsified, were observed for orange juices from different sources.

### EXPERIMENTAL

THE MAIN EXPERIMENTAL objective was to determine the effect of the concentration level of emulsified volatiles, in relation to the solubility, on the retention of volatiles of different solubility during freeze drying of aqueous solutions containing either 10% or 25% by weight dissolved sucrose (commercial grade). The volatiles chosen were d-limonene, n-hexyl acetate and acetone. d-Limonene is known to be the principal constituent of the essential oil of citrus juices, and is present at a level well above its aqueous solubility in those juices. n-Hexyl acetate was used to examine the retention of a substance with much higher solubility. It is also a food volatile, present in the aroma of apple juice and other substances. Acetone was chosen as a volatile with infinite miscibility. The solubilities and vapor-liquid equilibrium behavior of d-limonene and n-hexyl acetate were determined and are reported elsewhere (Massaldi and King, 1973).

#### Freeze-drying equipment

The apparatus is shown in Figure 2; it was essentially the same as that described previously by Bellows and King (1973) and Chandrasekaran and King (1972). As changes from those studies, a Welch Scientific Co., Model 1402 vacuum pump was used, and 40 gauge Chromel P-Constantan thermocouples fed to a recorder were used to monitor the temperatures of the sample near the top and bottom surfaces. Heat






-  Concentrate Regions (Dissolved Solids)
-  Ice Crystals
-  Interlinked Network of Voids, from Sublimation of Ice Crystals

Fig. 1—Structure of a liquid food or model solution during freeze drying.

<sup>1</sup> Present address: Departamento de Tecnología Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 y 115, La Plata, República Argentina

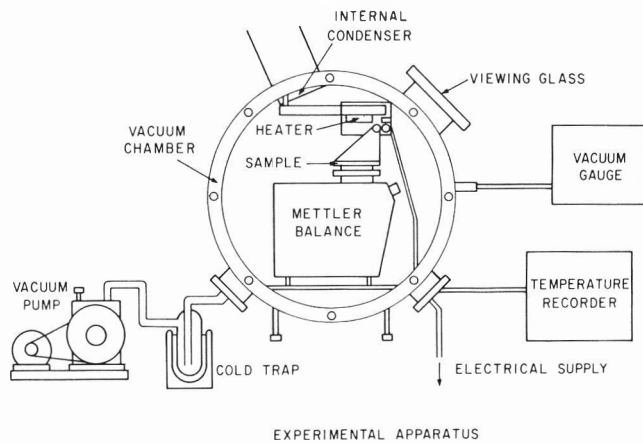


Fig. 2—Freeze-drying apparatus.

was supplied from above by means of a 60-watt radiant heater. The chamber pressure was measured with a NRC thermocouple gauge, and the weight loss was followed with a Mettler Corp. P1200 balance, on which the specimen was placed inside the chamber.

The sample holders were made of a stack of two plexiglas slides, 4 in. square and 1/16 in. thick, with a central 2 in. diameter hole. These slides were held to a 1/8 in. thick supporting bottom slide by means of bolts. The samples were frozen in place, inside the holes, and thus measured 1/8 in. high by 2 in. diameter. For runs where the volatile was added to the sample at room temperature or above, the sample holders were modified by adding a removable plexiglas cover, 2.5 in. square, to seal the system and guard against evaporation during the freezing step. The thermocouples were shielded, except for the section to be covered by the specimen which included the junction, and were attached to a sample holder by pressing them tightly between the slides. Thus the thermocouple wires ran laterally through the sample in planes

of nearly constant temperature. This, coupled with the small wire size, minimized both temperature distortions from conduction and any extra vapor escape along the leads between the slides.

**Materials and procedure**

A stock of sucrose solution was prepared and stored at  $-3^{\circ}\text{C}$ . Emulsions were prepared by taking 180 ml of the sucrose solution in a 250 ml flask, adding the necessary amount of volatile to give the approximate desired concentration and shaking vigorously for 1 min. The temperature of the solution was never higher than  $0^{\circ}\text{C}$  during this step. Emulsions prepared in this manner were proved to be stable in droplet size and concentration uniformity for more than 24 hr in the case of d-limonene and 8 hr for n-hexyl acetate.

One run was carried out using a solution initially saturated with the volatile, before freezing. In this case the equilibrium temperature for saturating the solution was  $40^{\circ}\text{C}$ , so that there would be a substantial change of solubility upon freezing.

**Slow-frozen samples.** Three numbered sample holders were tared and placed at  $-40^{\circ}\text{C}$  in a freezing chest, with thermocouples being attached to one of them. Nuclei to induce ice crystal growth were created by breathing upon the bottom of the sample holders. The model solutions, held just above the freezing temperature, were then poured into each holder through a side tube attached to the bottom of the 250 ml supply flask, so as to avoid any possible nonuniformity of the emulsion in the upper part of the flask. The sample holders were filled up to the level of the surface thermocouple and were left for 5–12 hr to allow for ice growth. Figure 3 shows the freezing curve observed for an initial sample temperature of  $40^{\circ}\text{C}$ .

Two blocks of dry ice were then placed on either side of each sample holder for 1/2 hr, after which the surfaces of all holders were scraped with a knife blade in two directions. These samples were rapidly weighed and then placed between the dry ice blocks again for another 1/2 hr. Shortly before starting the run the sample with the thermocouples was covered with a layer of dry ice powder and then placed on the balance inside the chamber. The thermocouples were connected, the chamber was sealed and the pump and timer were started.

During the run the background pressure, sample temperature and weight of sample were observed every 5 min. The background chamber pressure was typically  $10\ \mu\text{m Hg}$ . Figure 4 shows a typical recording of surface temperature vs. time, for freeze drying a 25% sucrose solution. The bottom temperature values followed very closely those shown for the surface temperature in Figure 4. Freeze drying started after 15–20 min, the time necessary for the ice front vapor pressure to overcome the background pressure. This point was noticed by lowering of the sample temperature following the disappearance of frost accumulated on the sample during the start up, as shown by the small maximum in Figure 4. Then the heat supply was started at an increasing rate, with the precaution of not abruptly heating the sample surface, upon which collapse of the semi-dried surface would occur. Finally, the heat input was set so as to fix the temperature

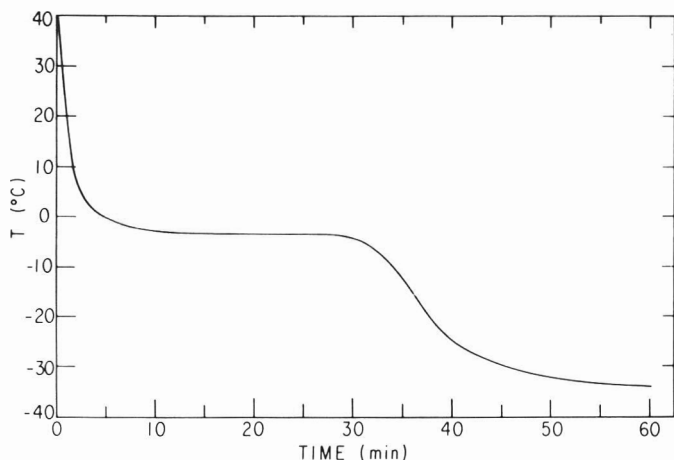


Fig. 3—Temperature-time relationship for a sample frozen from an initial temperature of  $40^{\circ}\text{C}$ .

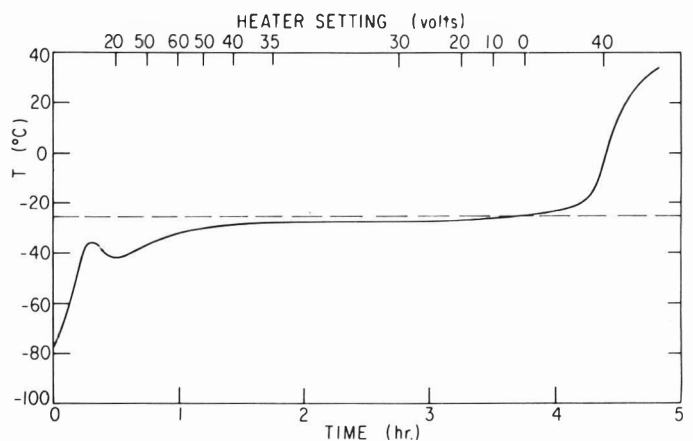


Fig. 4—Surface temperature history during freeze drying of a 25% sucrose specimen.

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4°C below the collapse temperature reported by Bellows and King (1973). Since the drying rate became increasingly mass-transfer limited towards the end of the run, the heater setting had to be slightly but continually decreased up to a point where the temperature increased rapidly, indicating that no more sublimation was taking place. At this point the heat supply was increased again to permit the removal of the last 2–5% of the moisture. When the surface temperature reached 30°C the run was terminated; this point corresponded to no further discernible weight loss taking place. The sample was removed from the chamber and carefully diluted in chilled water in the flasks to be used in the analysis.

The other two control samples were also dissolved in similar flasks to the same dilution and served as the measure of the volatile content of the specimens before freeze drying, to which 100% retention would correspond.

**Fast-frozen samples.** Three glass beakers, 2-in. diameter and 1/2 in. high were placed on a large container, which was filled with liquid nitrogen so as to cover half the height of the beakers. The model solution was then poured into the beakers up to about the same level as was used with the slow-frozen samples. The subsequent procedure was the same as that carried out for the slow-frozen samples, except that only the time required for handling and weighing the specimens was allowed for ice growth.

#### Analytical methods

The volatile solute content in the freeze-dried sample and in the control samples was measured by the technique described in the preceding paper (Massaldi and King, 1974), involving dilution with water to a point well below the solubility limit of the volatile followed by vapor headspace analysis with the flame-ionization chromatograph. Sucrose contents were determined with an Abbé Refractometer.

#### Microscopic observations

The drop-size characteristics of the emulsions were determined by observation of liquid samples of different concentrations under a Reichart Co., Zetopan research microscope, at

500X and 1250X magnifications. The field length was calibrated with a Petroff chamber, for both amplifications. It was not possible to measure the drop-size distribution quantitatively because of the high dilution of the emulsions, which presented only a few droplets in the field. However, an approximate droplet size could be determined by inspection of the sample on the slide at various points.

## RESULTS

IN FIGURE 5 are plotted the experimental results for d-limonene and n-hexyl acetate in 25% sucrose solutions. Also two data points are shown for acetone, an infinitely soluble volatile. The ordinate variable is the retention, defined as  $R = C_{av}/C_0$ , and the abscissa  $n = C_0/C_S$ .  $C_{av}$  is the average concentration of the volatile remaining after drying, referred to the initial (wet basis) amount of solution.  $C_0$  and  $C_S$  are the initial concentration and the solubility of the volatile. The correct temperature at which the solubility should be chosen is 0°C, the initial temperature of the emulsions if it is assumed that no growth of emulsion droplets occurs during freezing. Since no solubility data were found for n-hexyl acetate at 0°C, values at 25°C were used for both d-limonene and n-hexyl acetate so as to make the two analyses comparable. The effect of using values of  $C_S$  measured at 0° in Figure 5 would be a shift of the entire set of data somewhat to the right.

It is apparent from Figure 5 that the percentage retention in the emulsion system decreases with increasing concentration of the emulsion, up to a level at which an asymptotic retention is reached. This behavior would not be expected for the retention of a homogeneously dissolved volatile, which from the diffusion analysis should be nearly independent of

the concentration level of the volatile. It is also noticeable that the asymptotic value of retention is lower for n-hexyl acetate than for d-limonene, although for both the retention of the solute in homogeneous solution appears to be the same, as shown in the figure for  $n = C_0/C_S < 1$ . The retention level for acetone, which is homogeneously dissolved, is also consistent with this trend.

Figure 6 shows the same type of plot but for two different concentrations of sucrose, both with d-limonene as volatile. In this case,  $C_S$  is the solubility of d-limonene at 0°C in 25% (8.5 mg/liter) and 10% (9.2 mg/liter) solution, respectively. The same general behavior is observed for the 10% sucrose solution, but in this case the retentions at both low and high d-limonene concentrations are lower than for the 25% sucrose solution. Results are also shown for fast-frozen samples of a 25% sucrose model solution. The full curves drawn in Figures 5 and 6 correspond to the physical model discussed below.

The results of the microscopic observations are shown as representative photographs in Figures 7 through 11. Figure 7 shows a typical view of the emulsion for d-limonene at  $n = C_0/C_S = 2$ . The small, light spheres are droplets, with an average size of 1–2  $\mu\text{m}$ . Figure 8 shows the droplets observed at  $n = 12$ , where the range of drop sizes is about 5–16  $\mu\text{m}$ . Figures 9 and 10 show drop sizes for n-hexyl acetate at  $n = 2$  and 10, respectively, with the smallest discernible drops being about 5  $\mu\text{m}$  and the largest drops being about 7  $\mu\text{m}$  and 40  $\mu\text{m}$  for  $n = 2$  and 10 respectively. It is apparent that the drop size increases with increasing  $C_0$  for both volatiles. By comparing the drop sizes for n-hexyl acetate with those for d-limo-

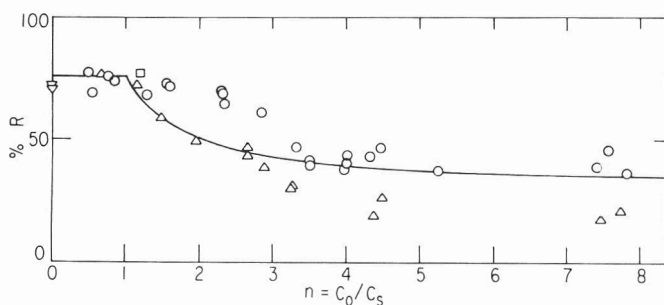


Fig. 5—Percent retention (%R) vs. level of volatile concentration, normalized by solubility at 25°C for 25 wt % sucrose solution. Volatiles:  $\circ$ , d-limonene;  $\triangle$ , n-hexyl acetate;  $\nabla$ , acetone, all emulsified at 0°C;  $\square$ , d-limonene, initially saturated at 40°C. Curve is the prediction of theoretical model (see Text).

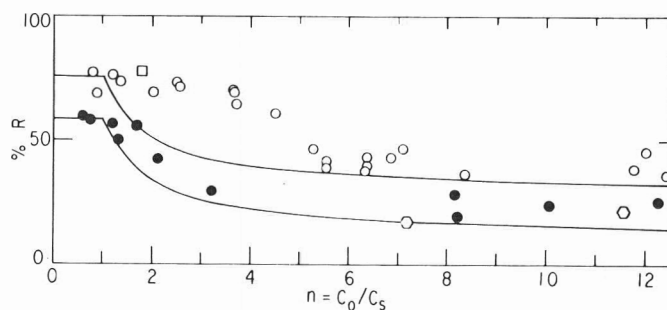


Fig. 6—Percent retention (%R) vs. level of d-limonene concentration, normalized by solubility at 0°C.  $\circ$ , 25 wt % sucrose;  $\bullet$ , 10 wt % sucrose, both frozen in freezer chest and emulsified at 0°C;  $\square$ , 25 wt % sucrose, frozen in freezer chest and initially saturated at 40°C;  $\circ$ , 25 wt % sucrose, fast-frozen and emulsified at 0°C. Curves are predictions of theoretical models for 25% and 10% sucrose (see Text).

nene, one can see that the drop size at a given  $n$  also increases with increasing  $C_S$ , although ultimately this is effected by having a higher  $C_O$  at a given  $n$  for the more soluble substance.

It was also found that sucrose concentration over the range considered did not have a detectable effect on the emulsion drop size.

A microscopic view of the freeze-dried product from a 25% sucrose solution is shown in Figure 11. The clear areas cor-

respond to the pores left by the ice after sublimation. The pores show an average characteristic diameter of about  $40 \mu\text{m}$ . The dark areas, connecting the pores, correspond to the interstitial concentrated sucrose solution.

DISCUSSION

Theoretical model

When a solution of a carbohydrate in water is cooled below the freezing point,

water segregates from the solution as ice, growing upon whatever nuclei are presented, leaving a concentrated amorphous solution (CAS) of a carbohydrate in the interstices. For a slab geometry, as used here, and unidirectional freezing, an appropriate idealization is to assume that ice grows in the form of parallel needles, perpendicular to the bottom of the holder, separated by a matrix of CAS of uniform dimensions. It is recognized that the situation is actually very different, as can

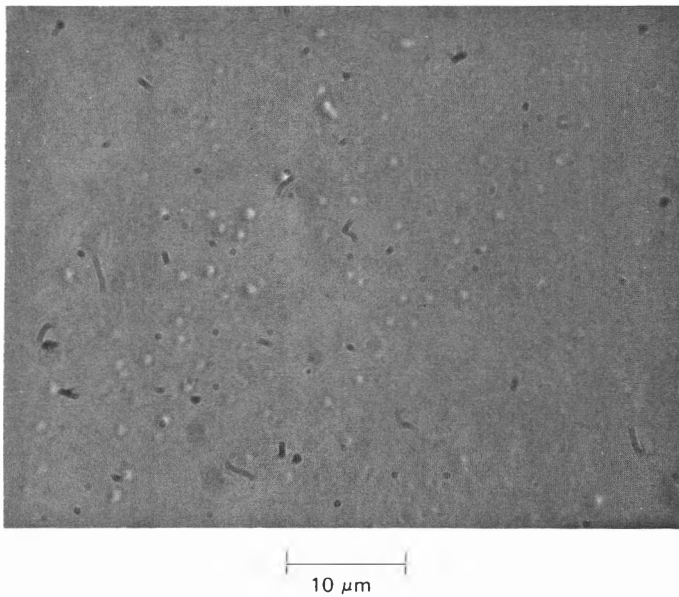


Fig. 7—Emulsion of *d*-limonene in 25% sucrose solution,  $n = 2$  (27.0 mg/liter). Field width =  $60 \mu\text{m}$ .

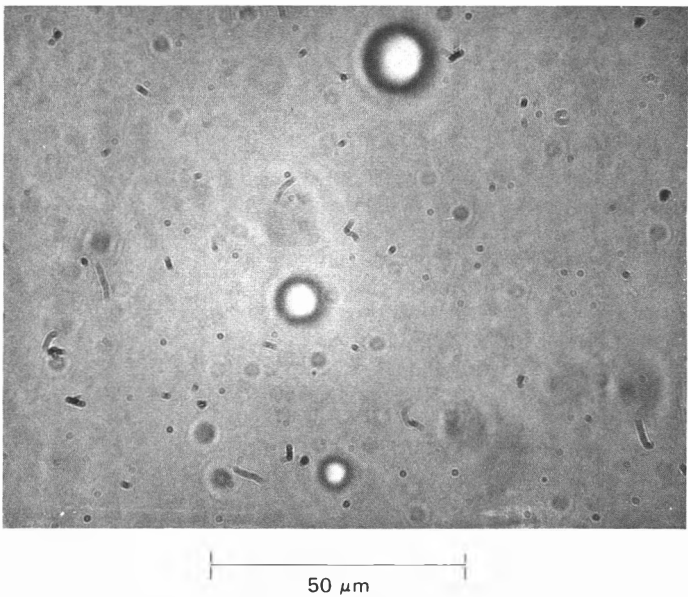


Fig. 8—Emulsion of *d*-limonene in 25% sucrose solution,  $n = 10$  (135 mg/liter). Field width =  $155 \mu\text{m}$ .

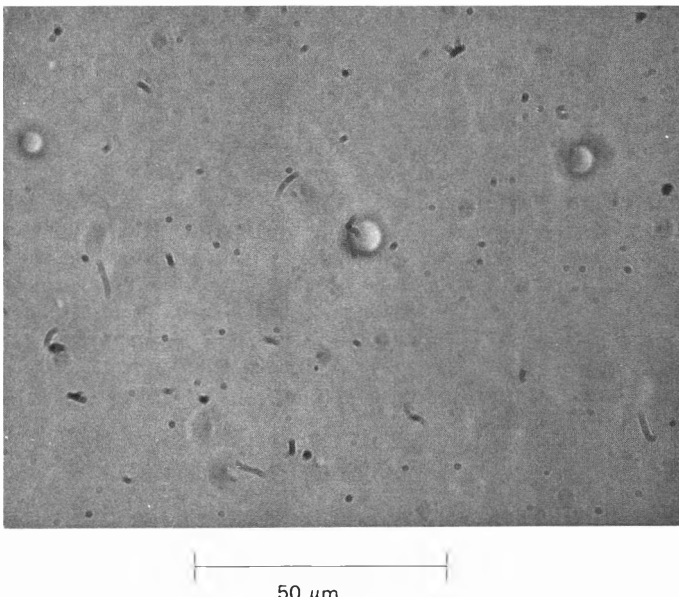


Fig. 9—Emulsion of *n*-hexyl acetate in 25% sucrose solution,  $n = 2$  (1924 mg/liter). Field width =  $155 \mu\text{m}$ .

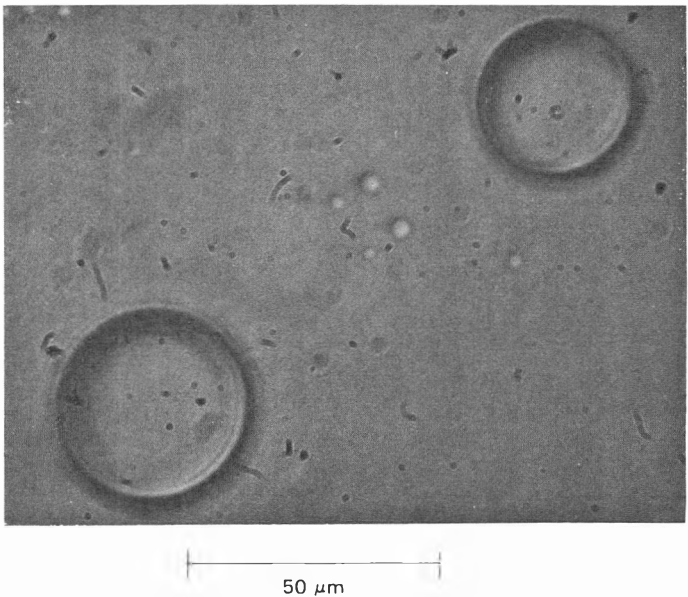


Fig. 10—Emulsion of *n*-hexyl acetate in 25% sucrose solution,  $n = 10$  (4620 mg/liter). Field width =  $155 \mu\text{m}$ .

be inferred from Figures 1 and 11. The ice needles do not grow parallel and are interconnected; thus the CAS matrix does not have uniform dimensions. However, this freezing model provides a useful picture to visualize other physical phenomena occurring in the CAS.

Since ice crystals incorporate water very selectively, all other substances initially present in the solution should remain in the CAS. Thus highly soluble volatiles, such as methanol or acetone, should be homogeneously dissolved in the CAS, but volatiles of only moderate solubility may become insoluble under the new conditions of high (60–70%) carbohydrate concentration and low temperature. Finally, volatiles of extremely low solubility which are present from the start as an emulsion should remain in the form of droplets in the CAS or at the phase interface.

At low temperatures and high concentrations of the CAS, molecular diffusion will no longer be able to maintain a uniform concentration within the interstitial CAS regions. A form of concentration polarization will develop, shown schematically in Figure 12. Since dissolved matter is rejected by the growing ice crystals, the CAS concentration at the interface ( $C_i$ ) becomes greater than the bulk-average concentration of the CAS ( $C_B$ ). When diffusion ceases to transport a significant amount of dissolved matter away from the interface, no further ice crystal growth will be possible. An estimate of the critical value of the diffusion coefficient for  $C_i$  to become significantly greater than  $C_B$  can be obtained by noting that the Fourier group  $Dt/L^2$  must be of order unity or less for the concentration polarization to develop. Here  $D$  is the solute diffusivity (e.g., for sucrose in water in the present work).  $L$  is the mean thickness of the interstitial webs of CAS, and  $t$  is the time allowed for freezing. From the pore diameters found in Figure 11 and from the calculated porosity of the dried sucrose samples,  $L$  may be estimated as  $13 \mu\text{m}$  for 25% sucrose solutions and  $2.5 \mu\text{m}$  for 10% sucrose solutions. Allowing freezing times of the order of 1 hr, one finds that the concentration polarization effect within the CAS should become important for conditions giving  $D$  in the range of  $10^{-13} \text{ cm}^2/\text{sec}$  or less. It is reasonable to expect values of  $D$  to become this low in the CAS at low enough temperatures, since the CAS viscosity during freeze-drying must be  $10^7$  cp. or greater (Bellows and King, 1973).

When ice is removed by sublimation, an open matrix of CAS is left, which will then lose water by evaporation. In order to be lost, volatiles within the CAS must somehow come into contact with the vapor-CAS interface. For dissolved vola-

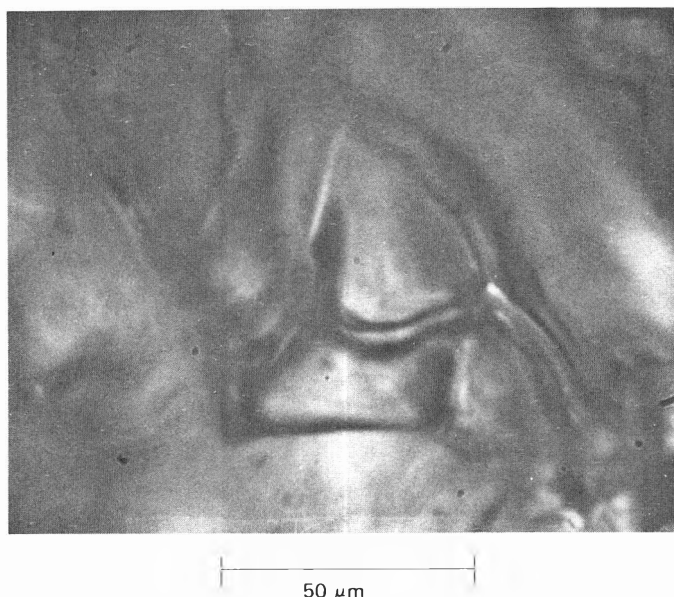


Fig. 11—Microscopic observation of freeze-dried sucrose, starting from 25% sucrose solution. Field width =  $155 \mu\text{m}$ .

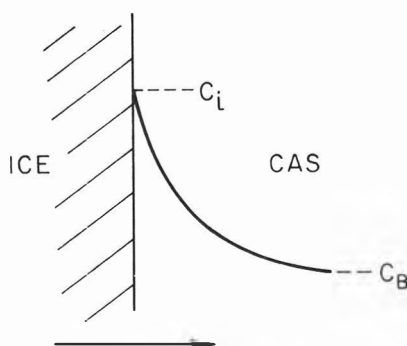


Fig. 12—Build-up of solute concentration adjacent to a growing ice-crystal interface.

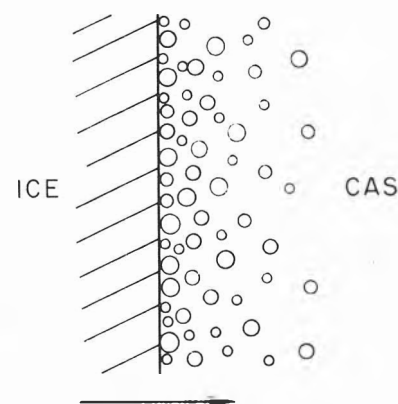


Fig. 13—Build-up of droplet concentration adjacent to a growing ice-crystal interface.

tiles this requires diffusion through the CAS, along a path that is increasingly concentrated in the dissolved solids, because of the concentration polarization and preferential drying of the CAS near the interface.

For undissolved volatiles, present in part as drops, the interpretation of the loss must also account for the presence of this second phase. Since the drops should not be incorporated into or occluded by the ice-crystal structure, they may be expected to build up in concentration near the CAS-ice interfaces in a manner analogous to the concentration polarization phenomenon for dissolved solutes discussed above. A schematic of the situation to be expected for the drops is

shown in Figure 13, where the number of drops per unit volume increases toward the interface. If the droplet diameter is less than the interstitial CAS thickness  $L$ , there exist two possibilities for the physical location of the drops. Either they are completely surrounded by the CAS, or else they are in contact with the ice interface. If the drops are large enough so that they have effectively a zero diffusivity, then basic diffusion theory indicates that they should not move relative to the surrounding fluid during the freezing process, as long as they are within the CAS. Drops initially within a region that becomes filled with an ice crystal during freezing would be pushed aside by the growing ice crystal. If



the change in molar volume of water upon freezing is neglected, then the proportion of the number of drops at interfaces to the number of drops entirely within the CAS should be the same as the overall volumetric proportion of ice crystals to CAS.

On the basis of this simple model, the volatiles retention can be predicted as the summation of the retention characteristics of the volatiles present in three different locations after freezing:

1. Droplets of volatile originally present within the regions eventually occupied by the ice crystals upon freezing. These droplets collect at the interface between ice and CAS, are therefore directly exposed to the vapor phase after sublimation of the ice, and are completely lost through vaporization.
2. Droplets of volatile originally present within the regions which remain as CAS after freezing. These are lost only through a relatively slow diffusion process, driven by the physical solubility of the volatile in the CAS. These droplets are therefore retained to a high degree.
3. Homogeneously dissolved volatile. This will be lost by diffusion within the interstitial concentrate, in the same way that diffusive loss occurs for substances below their solubility limit.

Table 1 shows the fraction of the total volatile which will be present in each of these locations (Column 1) after freezing, if  $\beta$  is the volume fraction of CAS and if  $n$  again is  $C_0/C_s$ . For simplicity it is assumed that volatiles initially in homogeneous solution before freezing remain in solution in the CAS rather than growing onto drops as supersaturation develops during freezing. Exploratory drop growth rate calculations indicated this assumption to be reasonable. Column 2 shows the fraction of each form of the volatile retained. In reality the diffusional processes governing the retention of drops and homogeneously dissolved volatile located within the CAS interact with

one another; however, for simplicity they are considered to be independent. Thus the retention of homogeneously dissolved volatile is considered to be  $f$ , the retention for a volatile which is below its solubility limit. The fractional loss for the drops in the CAS through diffusion is reduced by a factor  $n$ , since the concentration driving force for loss by diffusion of volatile through the CAS is  $C_s$ , while the amount of volatile present in this form is  $C_0$  ( $C_0/C_s = n$ ).

The overall retention of volatile is the sum of the entries in Column 3, which are the products of the entries in Columns 1 and 2. Thus the overall retention is given by,

$$R = (1 - \frac{1}{n})\beta (1 - \frac{1-f}{n}) + \frac{f}{n} \quad (1)$$

for  $n = C_0/C_s \geq 1$ . Equation (1) indicates that at high enough values of  $n$ , e.g.,  $n > 5$ , the main parameter governing the retention is  $\beta$ , which is the asymptotic value of  $R$  at large  $n$ . The model represented by Equation (1) does not distinguish between different volatiles since  $\beta$  is a parameter that depends only upon the freezing characteristics of the solution and thereby on the type and concentration of total solutes, but not on interactions between the volatile and the solution. The parameter  $f$  could vary somewhat from one volatile to another, but is not expected to be a major variable because of the similar diffusivities of different species in solution. On the other hand, the model does predict different asymptotes for different total solute concentrations before freezing, because of the change in the relative proportions of ice and CAS.

Estimation of parameters

The kinetically-limited freezing curve for sucrose-water solutions has been determined by Bellows and King (1973), using differential scanning calorimetry. Below a certain temperature, about  $-25^\circ\text{C}$ , the mass fraction of ice formed remains constant because of the concen-

tration polarization effect illustrated in Figure 12. The volume fraction of ice corresponding to any given temperature and any given initial sucrose content before freezing can then be found by simple application of a lever-rule mass balance to the kinetically-limited freezing curve, Figure 5 of Bellows and King (1973). For the freeze-drying conditions employed in this work,  $\beta = 0.29$  for 25% sucrose solution, and  $\beta = 0.11$  for 10% sucrose solution.

The value of  $f$  is difficult to predict *a priori*, but should be the magnitude of the retention at  $n < 1$ , and a relatively high value because of the known good retention of freeze-drying for homogeneously dissolved volatiles. From Figure 6,  $f$  was taken as 0.75 for 25% sucrose solutions and 0.57 for 10% sucrose solutions;  $f$  is expected to be lower for the lower dissolved solids content, on the basis of both experiment (Rulkens and Thijssen, 1972; Sauvageot et al., 1969; Flink and Karel, 1970) and theory (King, 1970; 1971). The value of  $f$  for acetone and n-hexyl acetate in Figure 5 agrees well with that for d-limonene, as is expected from a lack of major changes in diffusivity from solute to solute.

Comparison with experimental data

The full curves in Figures 5 and 6 show the predictions of Equation (1) with these values of  $f$  and  $\beta$ . The curves match the experimental value of the asymptote at high  $n$  well, predicting a somewhat lower retention than actually observed.

The retentions for n-hexyl acetate in Figure 5 are lower than both the data for d-limonene and the prediction from Equation (1). The explanation for this result probably lies in the size of the drops in the emulsion. In the case of n-hexyl acetate the ratio of drop diameter to the thickness of the webs of interstitial concentrate becomes greater than unity for a significant number of the drops (compare the drop sizes of 5-40  $\mu\text{m}$  from the microscopic observations with the estimated web thickness of 13  $\mu\text{m}$ ). This is not the case for d-limonene droplets, in which the maximum diameter is much smaller (16  $\mu\text{m}$  for  $n = 12$ ), although it does suggest that a few droplets may actually be forced to contact the ice-CAS interface due to a ratio of droplet diameter to web thickness greater than unity. These droplets are necessarily lost after sublimation of the ice. Thus the presence of drops of a size substantially greater than the CAS web thickness will decrease the retention below that predicted by Equation (1), as observed for n-hexyl acetate. The results for d-limonene retention with fast freezing shown in Figure 6 support this interpretation. Fast freezing gives smaller ice crystals, thinner webs, and consequently a greater

Table 1—Factors affecting retention of volatiles which exceed the solubility limit

Location of volatile upon freezing	Fraction of total initial volatile <sup>a</sup> (1)	Fraction of volatile retained (2)	Fraction of initial volatile retained (1 x 2)
Interface (initially in region occupied by ice)	$(1 - \frac{1}{n})(1 - \beta)$	0	0
Drops within CAS	$(1 - \frac{1}{n})\beta$	$1 - (\frac{1-f}{n})$	$(1 - \frac{1}{n})\beta [1 - (\frac{1-f}{n})]$
Homogeneously dissolved	$\frac{1}{n}$	$f$	$\frac{f}{n}$

<sup>a</sup> For  $n = C_0/C_s \geq 1$

ratio of drop diameter to CAS web thickness. For d-limonene in 25% sucrose solution this reduced the retention to the level observed for 10% sucrose solution.

Another discrepancy can be observed regarding the experimental data for d-limonene in Figure 6 in the range  $1 < n < 5$ . For 25% sucrose solution the experimental data do not fall as rapidly toward the asymptote with increasing  $n$  as is predicted by Equation (1). An explanation for this result lies in Brownian diffusion of the drops during freezing. The concentration of drops at the ice-CAS interface becomes greater than that in the bulk of the CAS during freezing, because of the rejection of drops from the growing ice crystals. As a result, Brownian diffusion will cause a net migration of drops into the CAS from the interface, giving a more-or-less continuous change in the number of drops per unit volume going from the interface to the bulk, as shown in Figure 13. This effect would increase the retention of volatile in drop form during freeze drying, as compared to the prediction of Equation (1) which postulates no diffusion of drops.

An estimate of the Brownian diffusivity ( $D_B$ ) of the drops can be obtained from the Stokes-Einstein equation (Daniels and Alberty, 1962):

$$D_B = \frac{kT}{6\pi R\mu} \quad (2)$$

where  $k$  is Boltzmann's constant ( $1.38 \times 10^{-16}$  erg/°K drop),  $T$  is temperature (°K),  $R$  is the drop radius, and  $\mu$  is the viscosity of the CAS. From Figure 7 the radius of d-limonene droplets in 25% sucrose at  $1 < n < 5$  is about  $1 \times 10^{-4}$  cm. From Bellows and King (1973),  $\mu$  at  $-3^\circ\text{C}$ , where most of the freezing occurs (see Fig. 3), is 5 cp ( $0.05$  dyne sec/cm<sup>2</sup>) for 25% sucrose. Substitution into Equation (2) gives  $D_B = 4 \times 10^{-10}$  cm<sup>2</sup>/sec.

As was mentioned above for dissolved solutes, the extent to which the drops diffuse into the CAS from the ice-CAS interface should increase with increasing values of  $D_B t/L^2$ , where  $t$  is the time allowed and  $L$  is the thickness of the CAS webs, becoming significant as  $D_B t/L^2$  approaches the order of unity. A typical freezing time, shown in Figure 3, is 0.5 hr. Using these values of  $t$  and  $D_B$ , along

with  $L = 13 \mu\text{m}$  for 25% sucrose solution when frozen, gives,

$$\frac{D_B t}{L^2} = \frac{(4 \times 10^{-10})(1800)}{(0.0013)^2} = 0.4$$

which is large enough for a significant Brownian diffusion of drops into the CAS to occur.

Following the same line of reasoning, the volume-average drop size becomes much greater for d-limonene at values of  $n > 5$ , as shown in Figure 8. By Equation (2), this decreases  $D_B$ , causing the group  $D_B t/L^2$  to become less than order unity and thereby giving better agreement with the prediction of Equation (1) at higher values of  $n$ . Similarly, for n-hexyl acetate the drop sizes are larger (Fig. 9 and 10), and the agreement of the n-hexyl acetate retention data (Fig. 5) with the prediction of Equation (1) at low values of  $n$  is better than for d-limonene. For d-limonene in 10% sucrose solution (Fig. 6) the average interstitial length  $L$  is less than for 25% sucrose. Thus even though small drops can diffuse at low values of  $n$  for 10% sucrose, the low value of  $L$  (about  $2.5 \mu\text{m}$ ) does not afford a sufficient diffusion path for the drops to escape the interface; this would cause the agreement of the observed retentions with the prediction of Equation (1) to be better for d-limonene in 10% sucrose than for 25% sucrose, as was found.

#### Extensions to other systems

The loss mechanism depicted by this model is probably also the explanation for the lower retentions found for less soluble components (n-hexanal and isovaleraldehyde) in the studies of freeze drying model solutions reported by Flink and Gejl-Hansen (1972). They observed small droplets in the freeze-dried matrix for those volatiles; these droplets would be a second phase composed of volatile substance which was present above its solubility limit. The lower retention for those volatiles is consistent with the predicted decrease in retention with increasing  $n$  as  $n$  exceeds unity.

Another interesting implication of the present interpretation of volatiles loss during freeze drying of heterogeneous systems is that the addition of an oil phase (e.g., coffee oil) before freeze dry-

ing may harm the volatiles retention. The oil phase can extract important aroma components from the aqueous phase. The oil with extracted aroma will be present at the ice-CAS interface and hence at the vapor-CAS interfaces in proportion to the volume fraction of ice during freezing, and thereby increase the vulnerability of those components to loss during freeze drying.

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- Reference to a particular product or brand name does not imply endorsement to the exclusion of others which may be suitable.

## RETENTION OF D-LIMONENE DURING FREEZE DRYING OF ORANGE JUICE

### INTRODUCTION

DRYING of citrus juices is becoming increasingly attractive in the food industry, yet large-scale application depends upon combining good product quality with low operating costs. Among others, factors affecting the quality of the dried product are thermal damage during the concentration and drying steps, aroma loss, and difficulty of redispersion. Because of the low temperatures involved and the highly porous structure of the dried product, freeze drying is one of the processes that most minimizes these effects.

Two drying processes have been used in recent works on volatiles retention in orange juice, foam-mat drying (Berry et al., 1972; Berry and Froscher, 1969) and freeze drying (Berry and Froscher, 1969; Sauvageot et al., 1969). No attempt has been made in these works to consider the retention of aroma components as a multi-phase problem. While freeze drying is potentially capable of rendering high retention of fully dissolved aroma compounds, this may not be the situation when the aroma is present in a second phase, as is pointed out in the preceding paper (Massaldi and King, 1974b).

The oil fraction of a citrus juice is a complex mixture of a large number of volatile substances, of which d-limonene constitutes 70–95% of the total. The aroma of the juice depends, however, on the presence of natural proportions of all aroma species. The presence of oil emulsion and colloidal matter (cloud) in citrus juices has been recognized for some time (Agricultural Research Service, 1956). More recently, mechanical separation of the different phases has been carried out (Peleg and Mannheim, 1970; Baker and Bruemmer, 1969; Scott et al., 1965; Mizrahi and Berk, 1970). Also the essential oil was determined in each fraction (Peleg and Mannheim, 1970) to show that the oil actually distributes between the aqueous phase and a separable solid phase.

It was the purpose of the present work to study the retention of essential oil, monitored as d-limonene, during the

freeze drying of orange juice. The results could then be interpreted in light of those obtained with model emulsions (Massaldi and King, 1974b).

### EXPERIMENTAL

THE FREEZE-DRYING apparatus used was the same as that employed in the preceding paper (Massaldi and King, 1974b). Microscopic observations were also performed in the same manner. The analytic technique for d-limonene determination was that reported by Massaldi and King (1974a). Samples of different types of orange juice from several sources were prepared by the procedure described by Massaldi and King (1974a):

- FS – Fresh squeezed juice (hand reamer)
- CP – Commercial pasteurized, single-strength juice
- CFC – Commercial frozen concentrate, reconstituted to strength indicated
- Serum I – Supernatant from CFC juice centrifuged for 30 min at 13,200 × G

Also CFC grapefruit juice, purchased locally and reconstituted to 25° Brix, was used. A fraction labelled Serum II, free of turbidity, was obtained by filtration of a portion of Serum I (from Brand E CFC juice) through Type MF Millipore® filter holder (a fritted Pyrex diaphragm) during a period of 48 hr. Table 1 summarizes the characteristics of the juice samples used. The oil level listed in this Table, deter-

mined by the headspace d-limonene analysis, is that of the juice as received except where indicated. The values of the lipid extraction parameter,  $\lambda_F$ , in Table 1 are taken from those given by Massaldi and King (1974a) for a 7-g sample weight diluted into 160 ml of liquid volume and 100 ml of vapor volume, and were assumed to hold for each type (FS, CP, etc.) of juice regardless of the source.  $\lambda_F$  represents the d-limonene absorptive capacity of the lipid fraction per unit weight and per unit dissolved solids content of the juice.

A portion of Navel FS juice was pasteurized by heating the sample in a closed vessel at 95°C for 2 min. Two fractions of this juice were subjected to further aging under different conditions: One fraction was agitated under moderate conditions with a magnetic stirrer at about 100 rpm in a closed flask at 25°C for 24, 48 and 72 hr, and the other one was kept unstirred at 5°C for 72 hr. These, and the rest of the nontreated samples, were stored at –5°C, no more than 48 hr before use.

The freeze-drying procedure followed closely the one described by Massaldi and King (1974b) for slow-frozen synthetic emulsions. The precaution was taken of maintaining the sample temperature below the particular collapse temperature of each juice, as reported by Bellows and King (1973), during the first stages of the drying process and throughout the run.

Redispersion of the dried product was fairly good, although a more sticky consistency was observed than in the case of sucrose solutions.

Table 1—Characteristics of samples of citrus juice used

Variety	Fruit	Type	Strength (°Brix)	d-Limonene Level (ppm)	$\lambda_F^a$
Navel	Orange	FS	10.8	61.7	9.9
Navel	Orange	FS	12.8	46.0	9.9
Valencia	Orange	FS	12.7	41.4	9.9
Valencia	Orange	FS	12.7	287.0 <sup>b</sup>	9.9
Brand A	Orange	CP	11.2	134.3	13.9
Brand A	Orange	CP	11.2	186.5 <sup>b</sup>	13.9
Brand B	Orange	CP	11.4	83.7	13.9
Brand C	Orange	CFC	10.0	92.0	5.9
Brand D <sup>c</sup>	Orange	CFC	25.0	308.0	5.9
Brand E	Orange	CFC	25.0	276.0	5.9
Brand E	Orange	CFC	10.0	115.0	5.9
Brand E	Orange	Serum I	25.0	156.0	3.9
Brand E	Orange	Serum II	25.0	173.0 <sup>b</sup>	0
Brand E	Orange	Serum II	25.0	317.0 <sup>b</sup>	0
Brand E	Grapefruit	CFC	25.0	176.0	5.9

<sup>a</sup> See Massaldi and King (1974a)

<sup>b</sup> d-Limonene added

<sup>c</sup> Partially synthetic

<sup>1</sup> Present address: Departamento de Tecnología Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 y 115, La Plata, Republica Argentina

**RESULTS**

RETENTION of essential oil, R, was defined as the ratio of the average concentration (wet basis) of d-limonene in the dried product to the initial concentration of d-limonene determined for the control samples, with all samples compared at the same dissolved-solids content, i.e.,

$$R = C_{av}/C_0$$

Figure 1 shows the experimental retention data for all types of juice studied, plotted versus the d-limonene level in the form of the dimensionless parameter  $n = C_0/C_s$ , where  $C_0$  is the initial concentration and  $C_s$  is the solubility of d-limonene in sucrose solution (Massaldi and King, 1973), referred to same % sugar, at 0°C. The curves drawn in this figure correspond to various physical models, and are discussed later. The range of values of n is much larger in this figure than in those shown for synthetic emulsions (Massaldi and King, 1974b), thus indicating that most of the data were obtained in the asymptotic high-n region of retention discussed in that paper. Symbols in Figure 1 correspond to different juice processing histories, without regard to juice strength, brand or oil concentration. Thus, data for CFC juices include strengths of 10 and 25° Brix, different brands and grapefruit, as well as orange juice.

The most striking feature of Figure 1 is the consistently high retention obtained for CFC juices of different strengths (above 90% in most cases), as contrasted with the low retention shown by the FS samples and for the synthetic emulsions of d-limonene studied in the preceding paper (Massaldi and King, 1974b). Furthermore, the synthetic emulsions showed different retention values for different dissolved solids contents in the high-n asymptotic region, which the CFC juices do not. Sauvageot et al. (1969) also found significant differences in the retention of d-limonene between the commercial and fresh juices used in their work, the higher retention corresponding to commercial juice. On the other hand, Berry and Froscher (1969) report retentions of essential oil between 25 and 50% in CFC orange juice during freeze drying, much lower than those found in this work.

In an attempt to ascertain the factors causing the observed differences in retention between the CFC juices, the FS juices, and the synthetic emulsions, different combinations of pasteurization and aging treatments were carried out with both types of juice prior to freeze drying, as indicated above. Thus, pasteurized FS juice and pasteurized, stirred FS juice show an improved retention with respect to nontreated FS juice, being highest for the stirred juice, as can be observed from

Figure 1. Also notice that a comparable, somewhat higher retention level, about 70%, was found for CP juices. On the other hand, pasteurized, not-stirred, aged FS juice shows a low retention, of the order of the nontreated FS juice.

Serum I and Serum II samples were both tested for retention. While Serum I shows the same high retention as the original CFC juice, Serum II shows a much lower one, indicating that the suspended and/or colloidal matter still present in Serum I, but removed in Serum II, is a very significant factor correlating directly with improvement of the retention of volatile essential oil in orange juice.

**DISCUSSION**

**The effect of cloud on retention**

One of the principal quality attributes of commercial citrus juices has been traditionally associated with what is called "cloud stability" (Agricultural Research Service, 1956; Scott et al., 1965; Baker and Bruemmer, 1971). From an empirical point of view, cloud can be defined as the particulate and/or colloidal matter which when suspended in the liquid imparts most of the consistency and flavor to the citrus juice. More precise definitions, as related to the separation achieved at dif-

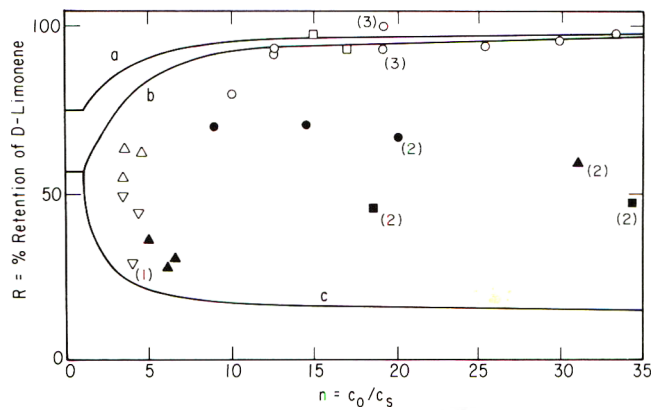


Fig. 1—Percent retention (R) vs. level of d-limonene concentration, normalized by solubility at 0°C; ○, CFC juices; ●, CP juices; ▲, FS juices; ▽, pasteurized FS juice; △, pasteurized, stirred FS juice; □, Serum I; ■, Serum II; (1) pasteurized, not-stirred, aged FS juice; (2) samples with d-limonene added; (3) grapefruit juice. Curves a, b and c are based upon theoretical models (see text).

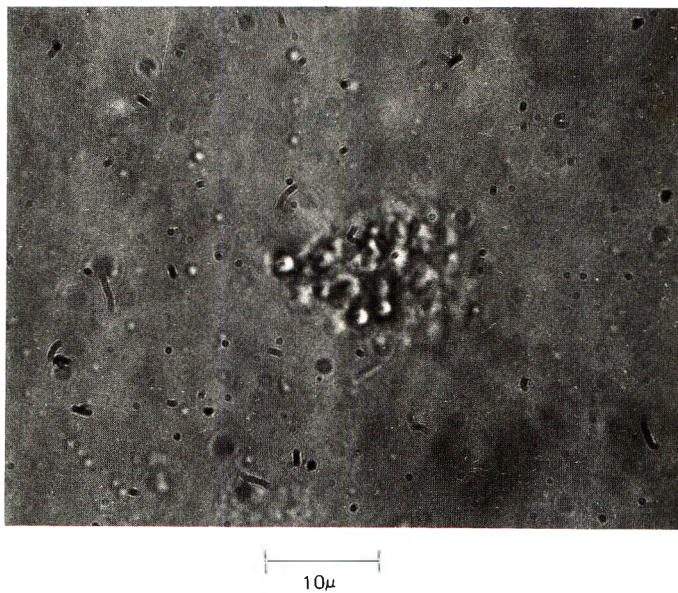


Fig. 2—Oil droplets attached to cloud particle, CFC juice. Field width = 60 μm.

ferent speeds in a centrifuge, are also used (Mizrahi and Berk, 1970). Much effort has been made to determine what substances compose the cloud and by what mechanism it can be stabilized. From the works of Scott et al. (1965) and Baker and Bruemmer (1969) the composition of typical cloud can be considered to be 25% lipids, 34% proteins, 33% pectins and 2.5% cellulose, plus hemicellulose and phosphorus (ca 1.5% as the pentoxide).

In the present work, the formation rather than the stability of the cloud is of more concern since in no case were conditions of temperature and time or storage allowed for an initially stable cloud to undergo instability for enzymatic or other reasons. The two phenomena, formation and stability of the cloud, are connected, however. Thus Loeffler (1941) reports that heating not only stabilizes but also enhances the density of the cloud. This fact would explain the beneficial effect of pasteurization upon d-limonene retention for FS juices found in the present work, which is not ascribable to enzyme inactivation. With regard to cloud formation, visual inspection of the different juices showed a distinct settling tendency of the suspended solids for FS and CP juices, whereas an homogeneous, dense and stable appearance was evident in reconstituted CFC juices.

Microscopic observations of CFC juices showed a high density of small particles, to which a number of oil droplets were attached. Figure 2 shows a photograph of this phenomenon. Oil droplet size can be estimated as 1 or 2  $\mu\text{m}$ . Notice also that other oil droplets are freely suspended in solution. Oil adhesion to cloud particles has also been observed by Mizrahi and Berk (1970). The droplet size they observed is about 1  $\mu\text{m}$ , in agreement with the value reported here. These features could not be observed in samples of CP and pasteurized FS juices. Instead isolated droplets of about the same size (1  $\mu\text{m}$ ) were observed, floating in a much clearer liquor. Correspondingly, the higher retention levels apply to the CFC juices, whereas lower retention levels were found for CP and pasteurized, stirred FS juices.

The correlation between cloud density and retention of d-limonene in freeze drying is evident in the various results obtained, with a denser cloud corresponding to improved d-limonene retention. This was confirmed by the experiments with Serum I and Serum II. The removal of cloud in Serum II greatly reduced the retention as compared to Serum I. The question then arises as to why the CFC samples show good cloud formation whereas CP and FS juices do not. The only differences that can be pointed out between them are that CFC juices have

been concentrated to a high strength in the processing steps, and have probably been subjected to a more severe combination of time and temperature.

#### Physical models

Simple, phenomenological models are developed in this section to serve as a basis for interpreting the results obtained with the different types of juice. This is done by considering the characteristics of the juice as related to cloud density and oil droplet size with the aid of the results obtained for d-limonene emulsions in the preceding paper.

The microscopically observed oil droplet size for CFC, CP and pasteurized, stirred FS juices was about 1  $\mu\text{m}$ , as mentioned earlier, independent of the oil concentration level. This represents a difference with respect to the behavior of the synthetic emulsions reported in the previous paper (Massaldi and King, 1974b), in which the drop size increased and the retention decreased with increasing concentration level,  $n$ , with the drop size becoming much larger than 1  $\mu\text{m}$  at high  $n$ . For the synthetic emulsions the retention observed for the smaller average droplet size (values of  $n$  between 1 and 5) was consistently higher than predicted by the model; this was attributed to Brownian-motion diffusion of droplets away from the advancing ice interface during the freezing step. Now, if the droplet size instead remains small and nearly constant as the d-limonene level increases, as was found in the microscopic observation of the juices, it is reasonable to expect that the retention in the emulsified-oil region of d-limonene concentration will be the same higher value, even at much higher values of  $n$ . This would then give a constant minimum retention level, higher than that corresponding to the case where the droplet size increases with  $n$ .

Another factor which can increase the retention with respect to synthetic emulsions is the presence of the lipid phase. This fraction can dissolve a substantial amount of oil, as can be inferred from the ratio of d-limonene in the lipid phase to that in the aqueous phase, given by Massaldi and King (1974a). For dilutions of the order of 20:1, the ratio of d-limonene in the lipid phase to that in aqueous phase was about 1.3:1. This ratio must be higher in the original juice due to a larger relative fraction of lipid phase. The dissolved oil present in the lipid phase can be expected to be lost only by a homogeneous diffusion mechanism and should therefore render a higher retention value than that found for droplets in the simple emulsions. However, drops were observed microscopically even at relatively low values of  $n$  ( $n \approx 5$ ). This indicates that retention of d-limonene dissolved in lipid is not the controlling mechanism, and indicates that at high values of  $n$  most of

the d-limonene must be present as droplets. Note also that the distribution coefficient  $K_1$  for d-limonene between the lipid and aqueous phases must therefore be less in a natural-strength juice than in a diluted juice. This would be expected as the oil level becomes larger in proportion to the lipid level.

The beneficial effects of pasteurization and stirring for retention in FS juices can therefore be explained mainly through a reduction of the average oil droplet size and, to a lesser extent, by the effect of heat and/or stirring on cloud formation or by a better dissolution of oil into the lipid fraction. Enzyme inactivation is not likely to be a factor, since conditions were not favorable for enzymatic action on the samples before use. In this way, a retention level comparable to, but somewhat lower than, that for CP juices was obtained. On the other hand, aging of an unstirred, pasteurized FS juice not only did not improve the retention with respect to the original nontreated FS juice, but it seemed to offset the favorable effect of pasteurization.

In order to explain the retention data for CFC juices, it is reasonable to search for some factor relating to the beneficial effect of a dense cloud. In the same way that sugar and droplets become more concentrated at the ice crystal interfaces due to the polarization originated by ice crystal growth (Massaldi and King, 1974b), cloud particles should also collect preferentially at the same interface. If the cloud density is high, these particles could form an effective barrier that impedes the contact of most of the oil droplets with the interface. This would greatly increase the overall retention since direct drop evaporation was found to be the main factor causing volatile loss in the case of synthetic emulsions. Then, by assuming as an extreme situation that oil droplets can never get in contact with the ice crystal interface, and ignoring the effect of oil dissolution into the lipid phase, the overall d-limonene retention can be predicted as the summation of the retention characteristics of d-limonene present in two different locations after freezing:

1. d-Limonene present in oil droplets. If droplets are never in contact with the ice crystal interface, d-limonene can be lost after sublimation of an ice crystal only through a homogeneous-solution diffusion mechanism, driven by its solubility in the interstitial concentrate.
2. Homogeneously dissolved d-limonene. This is lost by diffusion within the interstitial concentrate.

Table 2 shows the fraction of the total oil present in each of these locations under the assumptions made (Column 1) and the fraction of each form which is retained (Column 2). Again  $f$  is the frac-

tion retained for the homogeneously dissolved volatiles (Massaldi and King, 1974b). Other assumptions implied in this simplified model, as related to droplet growth and interaction of mechanisms, are the same as those discussed for the physical model in the case of synthetic emulsions (Massaldi and King, 1974b). Similarly, the overall retention can be obtained as the sum of the entries in the last column of Table 2.

$$R = \left(1 - \frac{1}{n}\right)\left(1 - \frac{1-f}{n}\right) + \frac{f}{n} \quad (1)$$

This equation differs from Equation (1) of Massaldi and King (1974b) by a factor  $\beta$  in the first term representing the different fate postulated for drops initially located in regions eventually occupied by ice crystals.

Equation (1) is drawn in Figure 1 with the values  $f = 0.75$  (Curve a) and  $f = 0.57$  (Curve b). These values of  $f$  correspond to 25 and 10% dissolved solids, respectively. It can be observed that both curves rapidly approach one another and that, as  $n$  reaches a high value, both curves approach 100% retention. Hence, this model not only closely reproduces the experimental data for CFC juices but also predicts nearly the same retention for juices of different strengths, as was observed experimentally.

Curve c in Figure 1 corresponds to the model developed for synthetic emulsions (Equation (1) of Massaldi and King, 1974b) with  $\beta = 13.9$ , corresponding to a 12.7% sucrose content. The data for non-treated FS juice seem to approach this line, thereby following the behavior observed with d-limonene emulsions. This suggests that the cloud-protective effect is absent and that the drops are large enough so as not to move by Brownian diffusion away from advancing ice-crystal surfaces.

Data for Serum I show no appreciable effect of coarser pulp removal on retention, thus giving a retention similar to that obtained for the original CFC juice. The removal of cloud in Serum II gives a product which, from the standpoint of retention, shows a behavior similar to that found for synthetic emulsions of same (25° Brix) strength (Massaldi and King, 1974b), allowing for the smaller drop size.

#### Comparison with other work

The essential-oil retention data reported by Berry and Froscher (1969), mentioned earlier, are much lower than those observed for d-limonene in this work for CFC juices. However, the experimental procedure and apparatus used in their work are different from those utilized here. Particularly, a much higher oil

Table 2—Factors affecting the retention of d-Limonene in CFC juices

Location of d-limonene upon freezing	Fraction of total initial d-limonene <sup>a</sup> (1)	Fraction of d-limonene retained (2)	Fraction of initial d-limonene retained (1 × 2)
Drops	$1 - \frac{1}{n}$	$1 - \frac{1-f}{n}$	$\left(1 - \frac{1}{n}\right) \left[1 - \frac{1-f}{n}\right]$
Homogeneously dissolved	$\frac{1}{n}$	$f$	$\frac{f}{n}$

<sup>a</sup> For  $n = C_0/C_s \geq 1$ .

level was used in their work, between 10 and 100 times the maximum used here. Although retention was found to be largely independent of oil level in the range covered in this work, orders-of-magnitude higher levels may have some effect on the retention. Thus, at high enough oil levels it is possible that bigger drops than those found in this work are formed, with the consequence of increasing the ratio of average droplet size to mean interstitial width of concentrate between the ice crystals. If this occurs, and if the postulated cloud-protective effect cannot counteract it, the retention will be lowered, as is discussed by Massaldi and King (1974b).

The results found in the present work are supported by those found by Sauvageot et al. (1969), who worked with CFC and FS juices and natural oil levels. It should be mentioned, however, that their results could possibly be affected by their not having accounted for the presence of the separate oil and lipid phases in their use of the headspace analysis technique, a problem discussed by Massaldi and King (1974a).

## CONCLUSIONS

UNDERSTANDING the retention behavior of volatiles during freeze drying of citrus juices requires a rather profound knowledge of the physico-chemical and biological characteristics of the food. The presence, formation and stability of dispersed phases and the distribution of volatiles between them seem to correlate with retention more than do the parameters usually considered in the works with homogeneous model solutions. Retention studies in other liquid foods should also be carried out from this viewpoint.

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## ANTHOCYANINS OF BEAUTY SEEDLESS GRAPES

## INTRODUCTION

ANTHOCYANINS impart an attractive red color to grapes and grape products. The amount and composition of the anthocyanins, among other factors, influence the shade and intensity of the color. Knowledge of the anthocyanins is important in the utilization of existing varieties and development of new varieties of commercial value.

A number of workers (Ribéreau-Gayon and Ribéreau-Gayon, 1958; Rankine et al., 1958; Albach et al., 1963; Akiyoshi et al., 1963; Somaatmadja and Powers, 1963; Liao and Luh, 1970) have investigated the anthocyanins of several *vinifera* grapes. Beauty Seedless is a purplish black *vinifera* grape of Scholokertek kiralynoje X Black Kishmish parentage (Brooks and Olmo, 1972) and the anthocyanins of this variety are unknown. Though Beauty Seedless grape was introduced in California in 1954, its commercial significance has been realized only recently. The increase in wine consumption in the United States and the high concentration of anthocyanins in Beauty Seedless prompted this investigation.

## EXPERIMENTAL

## Grapes

The Beauty Seedless grapes (16.8° Brix) were obtained in June, 1973 from the University of Arizona Experiment Station located at Tucson. The grapes were washed, stemmed and stored at  $-18^{\circ}\text{C}$  until used.

## Extraction

The grapes (500g) were blended with 0.1% conc HCl/MeOH (250 ml) in a Waring Blendor and filtered in a Buchner funnel through Whatman No. 1 filter paper. The residue was extracted twice with the same solvent. The combined filtrates were concentrated and purified by paper chromatography. All evaporations and concentrations were carried out in a rotary evaporator below  $35^{\circ}\text{C}$  under vacuum.

## Solvent systems

The solvent systems used for the purification and  $R_f$  value measurements are given in Table 1.

## Purification

The aqueous concentrated solution of pigments was purified by descending chromatography on several Whatman No. 3 MM papers ( $67 \times 23.3$  cm) in the following sequence: 1% HCl, BAW, AWH and BAW. The papers after each run were air dried. The bands were cut and eluted in a glass chamber by descending chromatography with 0.1% conc HCl/MeOH. The eluates were concentrated and the above procedure was repeated for each subsequent purification. The four-times purified pigments were sufficiently pure for the sugar, acyl, aglycone and alkaline degradation products determinations. A fifth purification using BAW was carried out for spectral measurements.

## Controlled hydrolysis

1 ml of 2N HCl was added to 1–2 mg pigment in MeOH (1–2 ml) in a test tube and the mixture was placed in a boiling water bath. At 0, 1, 2, 5, 8, 12, 20, 25, 30 and 35 min intervals, an aliquot was withdrawn and spotted on two Whatman No. 1 papers. The papers were developed with BAW and AWH. This test distinguished the type of glycosides. The monoglycosides give only two spots, namely, the monogly-

coside and the aglycone. The glycoside and aglycone spots are reversed in the aqueous and nonaqueous solvents.

## Aglycone and sugar

The pigment (3–5 mg) was hydrolyzed with 2N HCl (1–2 ml) by refluxing for 35 min in the same way used for partial hydrolysis. The mixture was cooled and the aglycone extracted with 1 ml amyl alcohol. The aqueous phase was extracted three times with 1 ml of 10% di-N-octylmethyl amine in chloroform to remove traces of HCl. Finally traces of amine were removed by extracting the aqueous solution of sugar with 1 ml chloroform. The sugar solution was dried under a stream of nitrogen, dissolved in a drop of water and spotted on two Whatman No. 1 papers along with authentic compounds (glucose, galactose, arabinose, rhamnose and xylose). The papers were developed independently in BAW and phenol. The air-dried chromatograms were visualized by spraying with aniline hydrogen phthalate (2.59g in 100 ml  $\text{H}_2\text{O}$ -saturated n-butanol) reagent followed by heating at  $105^{\circ}\text{C}$  for 10 min.

The amyl alcohol solution containing the aglycone was spotted on Whatman No. 1 papers and developed independently with BAW, Forestal and formic acid for  $R_f$  value measurements. For spectral measurements, the aglycone was purified on Whatman No. 3 MM paper using Forestal for development.

## Acyl moieties

1 ml of 2N NaOH was added to 2–3 mg pigment in MeOH in a test tube. The test tube was flushed with nitrogen, closed with a screw cap and let stand for 2 hr at room temperature. The mixture was then neutralized with an excess of 2N HCl (1.5 ml) and the acyl moiety was extracted three times with ethyl ether (10 ml each). The combined ether extracts were

Table 1—Solvent systems for paper chromatography

Solvent system abbreviation	Composition	Time of run (hr)	Solvent used for
BAW <sup>a</sup> -upper phase	n-butanol-acetic acid-water (4:1:5)	18	Anthocyanins, anthocyanidins, sugars and phenolic acids
BU-HCl <sup>b</sup> -upper phase	n-butanol-2N HCl (1:1)	18	Anthocyanidins
AWH	Acetic acid-water-conc HCl (15:82:3)	6	Anthocyanins and anthocyanidins
1% HCl	Water-Conc HCl (97:3)	4	Anthocyanins
Forestal	Acetic acid-water-conc HCl (30:10:3)	15	Anthocyanidins
Formic acid	Formic acid-conc HCl-water (5:2:3)	6	Anthocyanidins
2% HOAC	Acetic acid-water (2:98)	4	Phenolic acids
Phenol	Phenol-water (4:1)	15	Sugars

<sup>a</sup> BAW was used within 1–2 hr of mixing for  $R_f$  value measurements

<sup>b</sup> Paper equilibrated 24 hr after spotting and before running in the chamber containing aqueous phase

Table 2— $R_f$  values and color characteristics of alkaline degradation products<sup>a</sup>

Band no.	$R_f$ values X 100		Color with DPNA + Na <sub>2</sub> CO <sub>3</sub>	Identification
	BAW	2% HOAC		
1111	65	41	Grey-brown	Gallic acid
1211	76	49	Purple	3-O-Methyl gallic acid <sup>b</sup>
1311	85	57	Blue	Syringic acid
1321	88	60	Violet	Vanillic acid
1411	86	56	Blue	Syringic acid
1511	79	34	Brown	Caffeic acid
	85	56	Blue	Syringic acid
	88	40	Blue	p-coumaric acid

<sup>a</sup> Phloroglucinol ( $R_f$  values 0.68 and 0.61, respectively in BAW and 2% HOAC, and orange color with DPNA + Na<sub>2</sub>CO<sub>3</sub>) was detected in all the degradation products.

<sup>b</sup> Authentic compound was not used for comparison.

washed with distilled water and the ether evaporated under a stream of nitrogen. The residue was dissolved in a few drops of ether and spotted on two Whatman No. 1 papers along with authentic compounds (p-coumaric, ferulic and caffeic acids). The papers were developed independently with BAW and 2% HOAC. The air-dried chromatogram was visualized by spraying with diazotized p-nitroaniline (to 0.25g p-nitroaniline in 50 ml 2N HCl, add 5% NaNO<sub>2</sub> solution until colorless) reagent (DPNA). The color of the spots was intensified by spraying with 20% Na<sub>2</sub>CO<sub>3</sub> solution.

#### Alkaline degradation products

A saturated solution of barium hydroxide (2 ml) was added to the pigment (3–5 mg) in MeOH in a round bottomed flask. The mixture was refluxed for 1 hr under a stream of nitrogen in a boiling 10% brine bath. The mixture was cooled and neutralized with 2N HCl. The degradation products were extracted and analyzed in the same way as for the acyl moiety. The authentic compounds used were vanillic, syringic, protocatechuic and gallic acids and phloroglucinol.

#### Spectral measurements

The absorption spectra of anthocyanins and anthocyanidins in 0.01% conc HCl/MeOH were measured with a Perkin Elmer 202 UV-visible spectrophotometer. A solution obtained by eluting an appropriate blank area of the chromatogram with 0.01% conc HCl/MeOH was used as blank. The AlCl<sub>3</sub> shift was measured by recording the spectrum after the addition of 3 drops of 5% AlCl<sub>3</sub> in 95% ethanol. The UV observations were made under long wavelength UV light (Chromato-Vue CC-20, Ultra-Violet Products, Inc.).

#### Quantitative measurements

Two 50g samples of frozen Beauty Seedless grapes were hand peeled and the skins blended separately in a Waring Blendor with 50 ml 0.1% conc HCl/MeOH. The mixture was filtered in a Buchner funnel through Whatman No. 1 filter paper and the residue extracted twice with 25 ml of the same solvent. The combined filtrates were evaporated almost to dryness. The residue was quantitatively transferred to a 25 ml volumetric flask and made up to volume with 0.1% HCl/MeOH. After measuring the absorbance at the wavelength of maximum absorption (total pigments) an aliquot (2 ml) was streaked on Whatman No. 3 MM paper, and the paper was developed in BAW. The developed chromatogram was air dried and each band was cut and eluted with 0.1% HCl/MeOH in a glass chamber by descending chromatography. The eluates were quantitatively transferred to a 10 ml volumetric flask and made up to volume. After measuring the absorbance at the wavelength of maximum absorption (individual anthocyanins), aliquots of individual anthocyanins (5 ml) were mixed and the absorbance measured at the wavelength of maximum absorption (total anthocyanins). The concentrations were calculated based on the molar extinction coefficients compiled by Fuleki and Francis (1968) as follows:

$$\text{mg anthocyanin/100g fresh weight} = \frac{A}{E_{1\%}^{1\text{cm}}} \times \text{dilution factor} \times 1000$$

Where A = observed absorbance  
and  $E_{1\%}^{1\text{cm}} = \frac{\text{Molar absorbance} \times 10}{\text{Molecular weight}}$

Table 3— $R_f$  values and spectral characteristics of anthocyanidins from Beauty Seedless grapes<sup>a</sup>

Derived from Band no.	$R_f$ values X 100			Absorption AlCl <sub>3</sub>		Identification
	BAW	Forestal	Formic acid	max nm	Shift	
1111	46(42)	32(32)	13(13)	546(546)	+	Delphinidin
1211	53(52)	48(46)	23(20)	544(543)	+	Petunidin
1311	56(58)	60(60)	28(27)	542(542)	—	Malvidin
1321	73(71)	63(63)	31(30)	532(532)	—	Peonidin
1411	57(58)	60(60)	28(27)	542(542)	—	Malvidin
1511	57(58)	60(60)	28(27)	542(542)	—	Malvidin

<sup>a</sup> The values in parentheses are data reported by Harborne (1958, 1967).

## RESULTS

A PRELIMINARY examination of Beauty Seedless grape pigments by paper chromatography showed the presence of six pigments (designated as band nos. 1111, 1211, 1311, 1321, 1411 and 1511). None of the bands showed fluorescence under long wavelength UV light, indicating the absence of 3,5-diglycosides. Bands 1111 and 1211 turned blue after spraying with AlCl<sub>3</sub> reagent indicating the presence of free orthohydroxyl groups. The presence of acyl peaks in the spectra of bands 1411 and 1511 indicated that they were acylated.

Controlled hydrolysis of the purified pigments gave only two bands confirming that the anthocyanins were monoglycosides. Glucose ( $R_f$  values 0.17 and 0.40 in BAW and phenol, respectively) was the only sugar isolated from the anthocyanins. The acyl moieties in 1411 and 1511 were identified as caffeic acid and p-coumaric acid, respectively. The  $R_f$  values and color reactions of caffeic and p-coumaric acids are given in Table 2. The alkaline degradation products (Table 2) indicated that the aglycones were delphinidin (band 1111), petunidin (band 1211), peonidin (band 1321) and malvidin (bands 1311, 1411 and 1511).

The  $R_f$  values and spectral properties of anthocyanidins derived from Beauty Seedless pigments are given in Table 3. Based on the identification of anthocyanidins, sugar moieties, acyl moieties and alkaline degradation products, the anthocyanins of Beauty Seedless grapes were identified as delphinidin-3-glucoside (band 1111), petunidin-3-glucoside (band 1211), malvidin-3-glucoside (band 1311), peonidin-3-glucoside (band 1321), malvidin-3-glucoside acylated with caffeic acid (band 1411) and malvidin-3-glucoside acylated with p-coumaric acid (band 1511). The  $R_f$  values, color and spectral properties (Table 4) of the pigments confirm these identifications.

The amounts of individual anthocyanins in Beauty Seedless grapes are given in Table 5. Malvidin derivatives (malvidin-3-glucoside and acylated malvidin-3-glucosides) account for 73% of the total anthocyanins. The hand peeling of frozen



Table 4—R<sub>f</sub> values, color and spectral characteristics of anthocyanins of Beauty Seedless grapes<sup>a</sup>

Band no.	Color	R <sub>f</sub> values X 100				Absorption Max nm	Percent absorbance			AlCl <sub>3</sub> Shift
		BAW	BuHCl	AWH	1% HCl		UV max vis max	acyl max vis max	440 nm vis max	
1111	bluish purple	24	11	17	04	278,536	58	—	17	+
1211	bluish purple	33	17	24	03	278,536	53	—	20	+
1311	purple	39	15	33	06	273,536	49	—	20	—
1321	salmon pink	41	29	34	09	276,524	64	—	23	—
1411	purple	44	16	25	03	276,304,538	60	58	19	—
1511	purple	52	20	30	03	276,304,538	69	64	21	—

<sup>a</sup> The bands were identified as delphinidin-3-glucoside (1111), petunidin-3-glucoside (1211), malvidin-3-glucoside (1311), peonidin-3-glucoside (1321), malvidin-3-glucoside acylated with caffeic acid (1411) and malvidin-3-glucoside acylated with p-coumaric acid (1511), based on the data of Harborne (1958, 1967) and Liao and Luh (1970).

Table 5—The amount of anthocyanins in Beauty Seedless grapes

Band no.	Compound	Visible absorption max nm	E <sub>1cm</sub> <sup>1%</sup>	mg/100g Fresh wt
1111	Delphinidin-3-glucoside	536	559.3	2
1211	Petunidin-3-glucoside	536	242.3	7
1311	Malvidin-3-glucoside	536	524.4	23
1321	Peonidin-3-glucoside	524	218.8	6
1411	Malvidin-3-glucoside (acylated with caffeic acid)	538	420.3	10
1511	Malvidin-3-glucoside (acylated with p-coumaric acid)	538	420.3	12
	Total pigments (as malvidin-3-monoglucoside)	538	524.4	71
	Total anthocyanins (as malvidin-3-glucoside)	538	524.4	62

beauty seedless grapes avoids extraction of sugar and water. Freezing at -18°C toughens and loosens the skin which can be removed without the loss of pigments. This method, though tedious on a large scale, is comparable to the method of Chen and Luh (1967) where a large amount of solvent is required to elute the pigments completely from the ion-exchange resin.

DISCUSSION

BEAUTY SEEDLESS grapes contain high amounts of anthocyanins (62 mg/100g fresh grapes) of which malvidin derivatives (malvidin-3-glucoside and acylated

malvidin-3-glucosides) account for 73% of the total anthocyanins. Malvidin-3-glucoside has a relatively high molar absorptivity. Delphinidin, petunidin and malvidin-3-glucosides, and acylated malvidin-3-glucosides have the same absorption maxima (538 nm) in the visible spectrum. These factors contribute to the deep purple color of Beauty Seedless grapes.

The absence of 3,5-diglycosides in the Beauty Seedless grape anthocyanins is in agreement with the well-known fact that vinifera grapes contain only 3-glucosides. Beauty Seedless grapes have good potential for use as wine or juice grapes due to the high content of monoglucosides

which are more stable than the diglucosides (Robinson et al., 1966).

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## ETHYLENE INDUCED ISOPEROXIDASE CHANGES DURING FIBER FORMATION IN POSTHARVEST ASPARAGUS

### INTRODUCTION

ASPARAGUS SPEARS undergo texture changes that occur during maturation, either in the field or in storage, and these changes progress from the butt end of the stem to the tip. Toughening of asparagus is due to lignification of fibrovascular tissues and occurs within hours after harvest in spears stored at ambient conditions (Isherwood, 1963). Evidence points to the peroxidase catalyzed conjugation of phenylpropane units as an important control site of lignin biogenesis (Freudenburg, 1966; Siegel, 1957). The localization of peroxidase in the secondary and primary cell wall regions relates to lignin deposition (Helper et al., 1970) and evidence points to allotropic control of peroxidase in crown gall tissue culture (Lipetz and Garro, 1965) and sclereid cells of pear fruit (Ranadive and Haard, 1972). We previously showed that varietal and seasonal differences in lignification of pear sclereids was dependent on the association of peroxidase with cell walls which, in turn, was dependent on the ionic environment of the cell. Total peroxidase activity and isozyme profiles were not related to the degree of lignification in the developing pear. These findings led us to investigate the relationship between peroxidase association with cell walls and fiber formation in postharvest asparagus. Data presented here show that isoperoxidase change, not peroxidase cell wall association, relates to the onset of lignification in cut asparagus spears. Moreover, data are presented which indicate ethylene gas induces the isoperoxidase changes associated with fiber formation. Recently, Goldstein et al. (1972) reported that phenylalanine ammonia lyase activity is induced in asparagus by excision. They suggested that the rise in PAL activity relates to the onset of lignification as shown by previous studies (Rubery and Northcote, 1966).

### MATERIALS & METHODS

#### Source of asparagus

Spears (*Asparagus officinalis*, variety "Martha Washington") were hand harvested

with a knife at ground level and stored at 20°C in a 40 liter jar flushed with humidified air at approximately 10 liters per hour. Butt diameters ranged from 1 to 1.5 cm and spear length ranged from 20–30 cm. Spear size was uniform in comparative studies. Asparagus were also hand harvested at 5 cm below ground level and 5 cm above ground level for experiments to determine the influence of cutting height on fiber formation and ethylene evolution. After appropriate storage times, spears were divided into equi-weight thirds (butt, mid and tip sections), frozen in liquid nitrogen, and stored at -40°C prior to further analyses.

#### Peroxidase isolation

Individual asparagus spears were segmented into thirds which were equi-weight segments for the given spear. The three segments will be referred to as the "butt," "mid" and "tip" sections. The respective sections, from 2–6 spears, were thinly sliced into liquid nitrogen and the frozen tissue was powdered in a small stainless steel blender for four 15-sec intervals. A 10-g aliquot of powder was mixed with 25 ml of an isolation medium containing sucrose (0.4M), ascorbate (1%), L-cysteine (0.1%), calcium chloride (0.4M) and Tris-Cl (0.1M, pH 8.0). The slurry was squeezed through four layers of cheesecloth and centrifuged at 60,000 × G for 30 min at 4°C. The supernatant frac-

tion was collected and assayed as described below. In certain experiments the concentration of CaCl<sub>2</sub> in the isolation medium was varied from 0.0 to 1.0M to determine conditions of optimal release of peroxidase from cell particulates.

#### Peroxidase assay

Peroxidase (EC 1.11.1.7) was assayed by a method utilizing o-dianisidine as hydrogen-donor, hydrogen peroxide as oxidant and assay medium buffered with sodium maleate (0.1M, pH 6.0) according to Guilbault (1968). Absorbancy change at 460 nm was recorded during the initial 30 sec of constant velocity. Preliminary trials showed the pH optimum of this system was approximately 6.0.

#### Histochemical assay of peroxidase

Tangential sections of asparagus were incubated in a solution containing benzidine (0.001%), H<sub>2</sub>O<sub>2</sub> (0.003%) and sodium maleate (0.1M, pH 6.0). Development of blue color was followed using a binocular microscope. The washed residue fractions of peroxidase extracts were also assayed histochemically to judge the efficacy of peroxidase release from fibrovascular tissue.

#### Polyacrylamide gel electrophoresis

Peroxidase isozymes were resolved on polyacrylamide gels with Tris-glycine buffer (pH

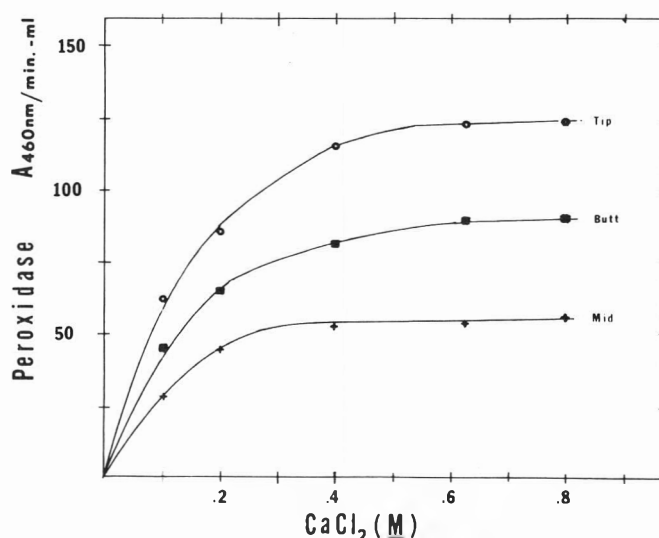


Fig. 1—Solubilization of peroxidase from freshly harvested asparagus spear tissue by addition of calcium chloride to the extraction medium. Spears were divided into tip (○—○), mid (+—+) and butt (■—■) sections prior to extraction. Data are for one experiment and are representative of three separate trials.

<sup>1</sup> Dept. of Food Science

<sup>2</sup> Dept. of Biological & Agricultural Engineering

<sup>3</sup> Dept. of Horticulture & Forestry

8.8) according to the method described by Ioannou et al. (1973). Electrophoresis was at 3 ma per tube for 2.5 hr in water jacketed electrode vessels maintained at 5°C. After electrophoresis gels were removed from the tubes and placed in an assay medium containing 0.05% benzidine and 0.02% H<sub>2</sub>O<sub>2</sub> in 10% acetic acid. The stained gels were transferred to 10% acetic acid and scanned (50 mm/min) at 460 nm with a gel densitometer attached to a Varian 635 spectrophotometer.

#### Analysis of ethylene

Spears (18–25 cm in length and 0.9–1.1 cm in diameter measured at 12.5 cm from the tip and) cut at 5 cm below ground, ground level and 5 cm above ground level were placed in 2 liter jars. The jars were kept at 20°C and ventilated with ethylene-free air at a flow rate of 200 ml/min. The evolved ethylene was collected in mercuric perchlorate for 10 min at 45, 95 and 165 min after cutting and subsequently released in sealed containers according to the method of Young et al. (1952). Gas samples were withdrawn from the sealed container and analyzed for ethylene by gas chromatography as previously described (Frenkel and Dyck, 1973).

#### Analysis of spear texture

The spear texture was measured using a shear press. For each spear, at every 1-in. interval, segments were used for cutting test using a 0.035-in. thick single-blade "Shear cell." The blade speed was adjusted to 1.0 in./min for all the tests. Force-deformation curves were recorded for each spear segment using a universal testing machine and x-y plotter. The maximum value on the force scale designated as "peak cutting force" was taken as a measure of toughness of the segment.

## RESULTS & DISCUSSION

### Peroxidase solubilization from fibrovascular tissue

Histochemical examination of peroxidase in asparagus revealed that the enzyme was predominantly localized with the fibrovascular bundles. Tissue extracted with buffer containing no added CaCl<sub>2</sub> contained negligible peroxidase in the supernatant fraction. Addition of calcium chloride to the isolation medium resulted in a progressive release of peroxidase from the particulate to the soluble fraction (Fig. 1). Optimal release of peroxidase was achieved at approximately 0.4M CaCl<sub>2</sub> in all three sections of the spear. Centrifugal pellets of tissue extracted with buffer containing 0.4M CaCl<sub>2</sub> were washed with a second aliquot of isolation medium and shown to contain negligible peroxidase activity associated with the particulate fraction. The concentration of CaCl<sub>2</sub> required to elicit optimal solubilization of peroxidase from asparagus aged for 72 hr at 20°C after harvest, was identical to that shown in Figure 1 for fresh spears.

### Changes in peroxidase activity of harvested spears

The total activity of peroxidase recovered from the butt, mid and tip sec-

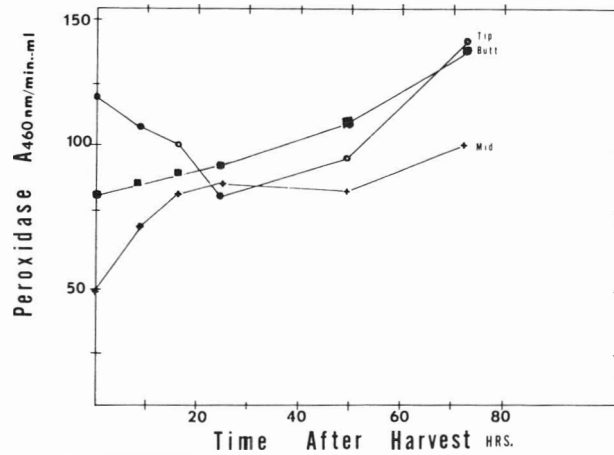


Fig. 2—Recovery of total extractable peroxidase from spear tissue stored at 20°C for up to 72 hr. Spears were divided into tip (○-○), mid (X-X) and butt (■-■) sections prior to extraction with medium containing 0.4M CaCl<sub>2</sub>. Results are average of three separate experiments.

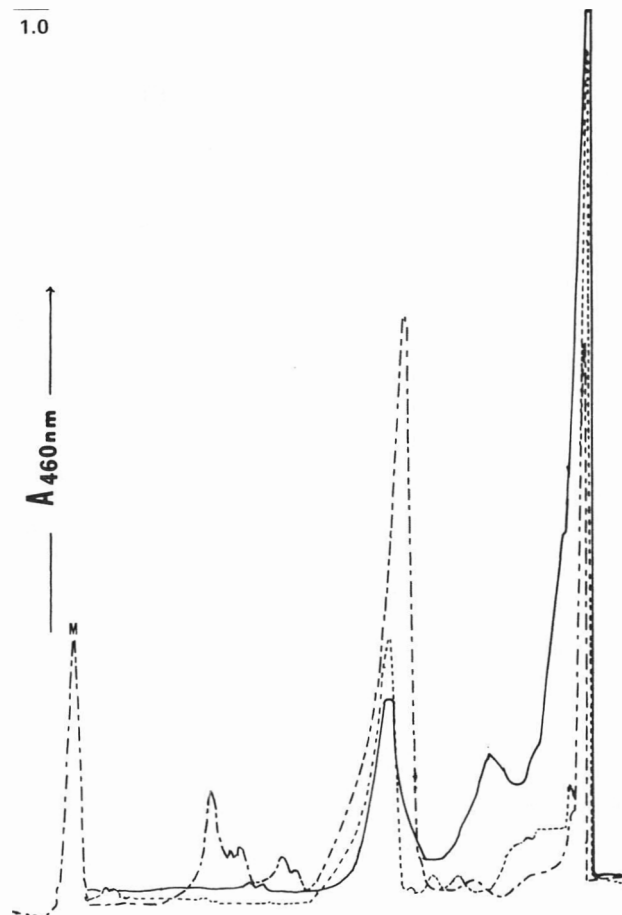


Fig. 3—Densitometric scans (460 nm, 50 mm/min) of polyacrylamide gels of 10 $\mu$ l peroxidase extracts from spears frozen in liquid nitrogen immediately after harvest. Spears were divided into tip (---), mid (---) and butt (—) sections prior to extraction with medium containing 0.4M CaCl<sub>2</sub>. Marker dye (M) was approximately 8 cm from the origin of the gels.

tions of freshly harvested spears was different (Fig. 1, 2). The tip end with its floral head contained approximately 2.5 times more activity than the adjacent mid-section of the spear. Histochemical examination revealed that the bulk of peroxidase associated with fresh tip segments was localized in the floral parts. Peroxidase levels in the butt sections of fresh spears were intermediate between those in the mid and tip sections. Recoverable peroxidase was observed to change immediately after harvest, although not in the same direction for the three sections of the spear (Fig. 2). Activity from the tip declined during the initial 24 hr postharvest while peroxidase in the mid and butt sections increased gradually over the entire 73 hr period of storage. The rapid decline in activity observed in tip sections would appear to relate to the disappearance of specific isozyme species while the increase in total activity in the butt, mid and tip (after 24 hr) was coincidental with the emergence of new isozyme species (see below).

#### Isoperoxidase distribution

The profiles of peroxidase isozymes from the three segments of fresh spears are shown in Figure 3. While the spectra of isozymes in the three segments differed somewhat, the species with  $R_f$  of 0.42 and 0.03–0.05 were predominant in all three sections. The zone having  $R_f$  0.42 was most intense in the tip section ( $A_{460\text{ nm}} = 0.67$ ), intermediate in the mid ( $A_{460\text{ nm}} = 0.35$ ) and lowest in the butt section ( $A_{460\text{ nm}} = 0.20$ ). Alternatively the zone with  $R_f$  0.03–0.05 was lowest in the tip ( $A_{460\text{ nm}} = 0.64$ ), intermediate in the mid ( $A_{460\text{ nm}} = 1.15$ ) and most pronounced in the butt ( $A_{460\text{ nm}} = 1.45$ ). Several anionic species ( $R_f > 0.5$ ) were present only in the tip section of fresh spears. The reciprocal relation between zones with  $R_f$  0.03 and  $R_f$  0.42 should be noted since this trend continued during the initial stages of aging.

Gel profiles of the three segments after 8 hr storage at 20°C showed the same

basic pattern of isozymes as the fresh cut spears with the following changes in quantitative distribution. The zone with  $R_f$  0.42 decreased in the three sections as follows: tip ( $A_{460\text{ nm}} = 0.67 \rightarrow 0.40$ ), mid ( $A_{460\text{ nm}} = 0.35 \rightarrow 0.29$ ) and butt ( $A_{460\text{ nm}} = 0.26 \rightarrow 0.18$ ) while the zone with  $R_f$  0.03–0.05 increased in the tip ( $A_{460\text{ nm}} = 0.64 \rightarrow 1.1$ ) and decreased slightly in the mid and butt sections. Also the  $A_{460\text{ nm}}$  of zones having an  $R_f$  intermediate between these two zones increased most dramatically in the butt section and to a lesser extent in the mid and tip sections.

Figure 4 shows isoperoxidase distribution in spear segments after 24 hours aging at 20°C. The isozyme zones with  $R_f$  between 0.15 and 0.5 showed a continued increase and as in 8-hr samples, these changes were most pronounced in the butt segments and least pronounced in tip segments.

After 36 hr storage the isoperoxidase zones with  $R_f$  ranging from 0.1–0.5 showed continued increase which was

1.0

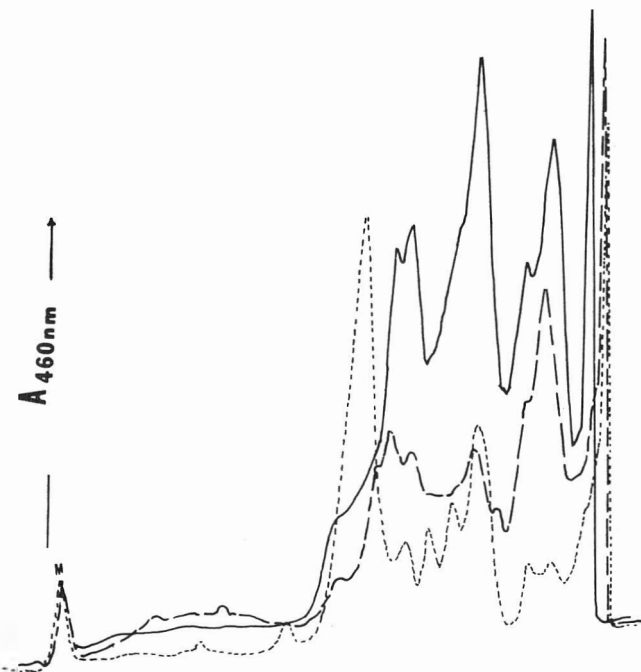


Fig. 4—Densitometric scans (460 nm, 50 mm/min) of polyacrylamide gels of 10  $\mu$ l peroxidase extracts from spears stored at 20°C for 24 hr after harvest. Spears were divided into tip (---), mid (···) and butt (—) sections prior to extraction with medium containing 0.4M  $\text{CaCl}_2$ . Marker (M) was approximately 8 cm from the origin of each gel.

2.0



Fig. 5—Densitometric scans (460 nm, 50 mm/min) of polyacrylamide gels of 10  $\mu$ l peroxidase extracts from spears stored at 20°C for 48 hr after harvest. Spears were divided into tip (---), mid (···) and butt (—) sections prior to extraction with medium containing 0.4M  $\text{CaCl}_2$ . Marker (M) was approximately 8 cm from the origin of each gel.

most pronounced from the butt end and finally after 48 hr the tip, mid and butt sections all exhibited substantial activity in isozyme zones with  $R_f$  ranging from 0.1-0.5 (Fig. 5). While the patterns of isozymes in the three sections showed clear differences in relative distribution after 48 hr, the trend of change to this point in time was quite consistent. That is, with time there was a progressive emergence of some 8-10 isozymes spe-

cies with  $R_f$  values ranging from 0.1-0.5. These changes were preceded by a rapid decline in the zone with  $R_f$  0.42 (Fig. 1) and a concomitant increase in the low  $R_f$  zone (Fig. 1). These trends are depicted graphically in Figures 6 and 7.

**Influence of exogenous ethylene on isoperoxidase change**

Application of 100 ppm ethylene to freshly harvested spears for 1 hr and sub-

sequent storage at 20°C resulted in an acceleration of the emergence of isozyme zones with  $R_f$  ranging from 0.1-0.5. The effect was most pronounced in the tip segments. That is, the progression of emergence of these isozymes was more uniform throughout the spear. Spears stored for 24 hr after ethylene exposure show comparable isozyme profiles as those stored for 48 hr without exposure to exogenous ethylene.

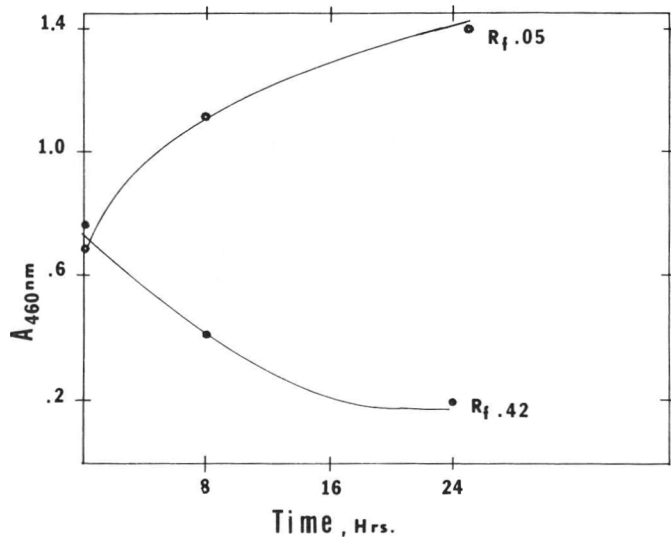


Fig. 6—Change in peak height ( $A_{460\text{nm}}$ ) of electrophoretic zones having  $R_f$  0.42 and  $R_f$  0.05 on gels of extracts from spears (tips) stored for up to 24 hr at 20°C. This trend, observed in tip sections, appeared to be more advanced in mid and butt sections.

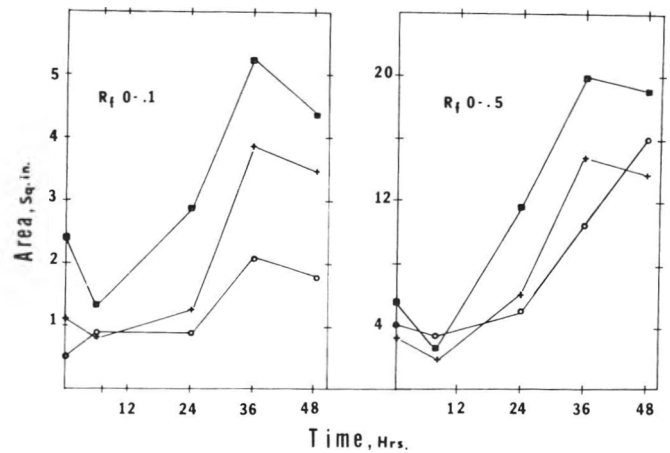


Fig. 7—Area in square inches of densitometric scans with  $R_f$  0.0-0.1 and  $R_f$  0.0-0.5 as a function of storage time at 20°C. One inch represents  $A_{460\text{nm}}$  of 0.1 and approximately 1 cm distance along the gel. Tip (○-○), mid (X-X) and butt (■-■) sections, respectively, showed increased lag in emergence of isozyme species with the indicated  $R_f$ .

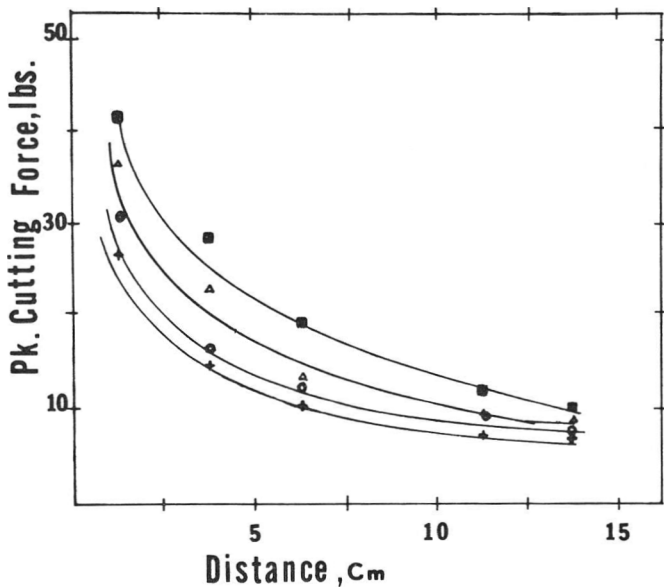


Fig. 8—Effect of aging time on toughness as measured by cutting force. The spears were 22.5-24 cm long and were stored at 10°C for 2 hr (+-+), 4.5 (○-○), 10 hr (△-△) and 72 hr (■-■). Distance of measurement refers to distance from ground level at time of harvest. Each data point represents an average of readings from four spears.

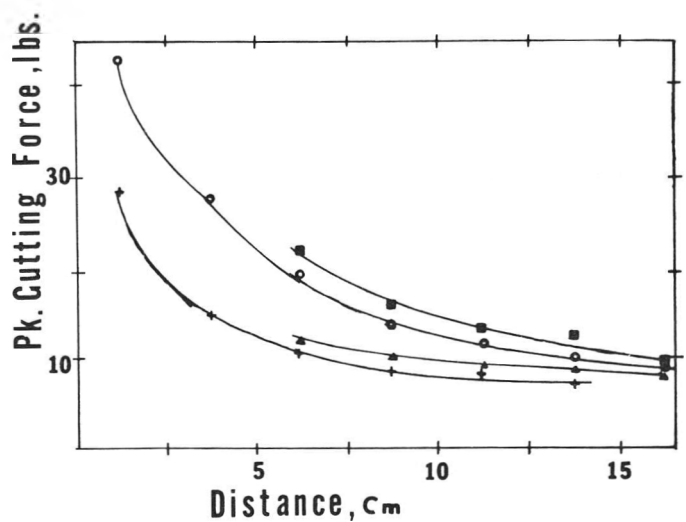


Fig. 9—Effect of height of cut on the rate of toughening of spears stored at 10°C for 2 hr (△-△; +--+ ) and 72 hr (■-■; ●-●). Spears were cut at 5 cm above ground level (■-■; △-△) or at ground level (○-○; +--+). Each data point represents an average of readings from four spears.

Table 1—Ethylene evolution by freshly harvested asparagus

Time after cutting (min)	Height of cut (cm)	Ethylene evolution	
		$\mu\text{l/Kg-Hr}$	$\mu\text{l/Hr-spear}$
45	-5	2.05	0.0486
	0	2.13	0.0400
	+5	3.71	0.0532
90	-5	2.61	0.0620
	0	3.13	0.0590
	+5	4.91	0.0702
165	-5	2.61	0.0020
	0	3.12	0.0688
	+5	5.51	0.0788

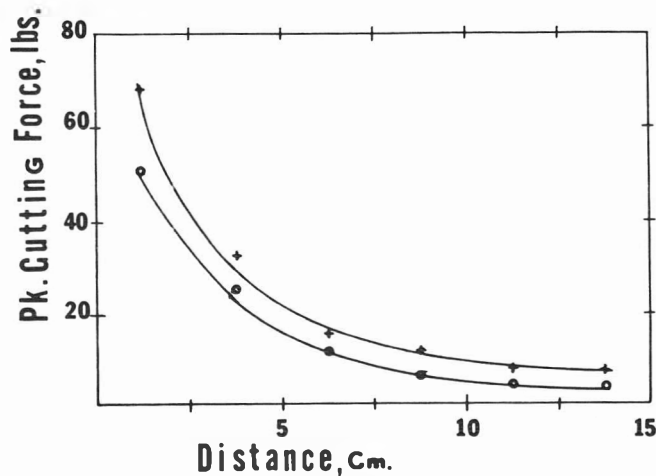


Fig. 10—Influence of ethylene treatment on subsequent toughening of spears held for 9 hr at 25°C. Ethylene treated (+—+) and control spears (o—o). The same trend was obtained for spears stored at 4°C.

#### Fiber formation in asparagus spears

The postharvest toughening of aging asparagus was evaluated by "peak cutting force." The toughening of spears, as judged by "peak cutting force," over 2–72 hr at 10°C is shown in Figure 8. It can be seen that spear toughness increased with aging time and decreased along the axis of the spear. The change was rapid during the first few hours after harvest and slowed down thereafter. The toughening in the spear was dependent upon the cutting height at harvest (Fig. 9). Spears cut at ground level did not toughen as rapidly as those cut 5 cm above ground level.

Treatment of harvested spears with air containing 100 ppm ethylene for 1 hr prior to storage at 25°C for 9 hr was shown to accelerate toughening of the spears (Fig. 10). The relative increase in toughening was more pronounced in the tip segment, thus resulting in a more or less uniform increase in toughness throughout the spear. The nature of the

increase in toughness caused by ethylene was similar to the observed increases in isoperoxidase species.

#### Ethylene evolution by cut asparagus spears

In that exogenous ethylene was observed to stimulate both isoperoxidase shifts and spear toughening, it was of interest to examine ethylene evolution from spears cut at different positions from ground level. The data in Table 1 show that ethylene evolution was most pronounced in spears cut above ground level. This observation is consistent with the fact that spears toughen more rapidly when cut at points above ground.

#### CONCLUSIONS

WE HAVE OBSERVED that dramatic changes in isoperoxidase distribution occurs in postharvest asparagus. New isozyme species appear initially at the butt end and finally at the tip end of the spear. The rate of isoperoxidase change which occurs during aging at 20°C is

accelerated by exposure of spears to exogenous ethylene. Moreover, the development of fiber in asparagus was also accelerated by exogenous ethylene and the increase in rate of fiber development in spears cut at higher levels above ground can be accounted for by the more pronounced ethylene evolution by spears cut above ground level. In view of the well known role of peroxidase in lignin biogenesis it is attractive to suggest that wound ethylene initiates a shift to peroxidase isozymes which are functional in lignification. This view is supported by the fact that storage of spears under elevated levels of carbon dioxide results in minimal fiber development (Lipton, 1965). Carbon dioxide is a competitive inhibitor of ethylene mediated processes (Burg and Burg, 1967). More data are needed to verify the thesis that wound ethylene initiates lignin deposition in harvested asparagus spears. It would appear that other factors, such as oxygen availability, the cellulosic matrix and the supply of phenylpropanoid residues, may also limit spear toughening under a given set of circumstances.

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## KINETICS OF THE THERMAL DEGRADATION OF METHYLMETHIONINE SULFONIUM IONS IN CITRATE BUFFERS AND IN SWEET CORN AND TOMATO SERUM

### INTRODUCTION

THE EVOLUTION of dimethyl sulfide (DMS) from various foods has been shown to arise from the thermal decomposition of methylnmethionine sulfonium salts (MMS) which are naturally present in the raw food. These salts were first isolated by Challenger and Hayward (1954) and by McRorie et al. (1954). Upon heating the isolated MMS and synthetic MMS in aqueous solution, the salts decomposed to form homoserine and evolved the characteristic odor of dimethyl sulfide. Skodak et al. (1965) determined MMS in several different foods using an automated amino acid analysis procedure. By heating a tomato sample for 1 hr at 100°C, the production of homoserine corresponded to the disappearance of MMS on an equimolar basis. Wong and Carson (1966) also isolated MMS from tomatoes using various adsorption and precipitation steps. Similar procedures were used by Keenan and Lindsay (1968), Bills and Keenan (1968) and Werner et al. (1969) in isolating MMS from milk, sweet corn and potatoes. The isolated MMS produced homoserine and DMS when heated.

Toennies and Kolb (1945) indicated that the pathway of decomposition of MMS was related to the acidity of the medium. Lavine et al. (1954) later reported two reactions that MMS could undergo depending upon the conditions of acidity. Heating in strong acid decomposed the salt to form methionine; however, heating the salt in water or alkali resulted in the formation of homoserine and DMS. Refluxing a solution of methylnmethionine sulfonium bromide (MMSBr) in water at pH 4.0 for 1 hr led to 64% decomposition of the salt with the formation of DMS and homoserine while complete decomposition occurred in two equivalents of alkali. The purpose of this investigation was to study the reaction characteristics of MMSBr in sodium citrate buffers at various pH's and temperatures and to compare these results with similar data from sweet corn and tomato serums.

### EXPERIMENTAL

#### Reaction procedure

Sodium citrate buffer (0.2M) solutions were prepared at pH 4.0, 5.0, 6.0, 7.0 and 8.0 to study the thermal degradation reaction of MMSBr. Each buffer solution (10.5 ml) was pipetted into culture tubes (16 × 100 mm) sealed with Teflon-lined screw caps. Eight tubes containing the buffer solution were placed in a water bath and allowed to equilibrate to 82°C. 0.5 ml of MMSBr stock solution, prepared by dissolving known quantities of MMSBr (Schwarz/Mann) in the various pH buffers, was added to each tube. After 1 min equilibration reaction timing was begun. Duplicate tubes were removed after 20, 40, 60 and 80 min reaction time. The reaction was stopped by placing the tubes in an ice-water bath. Using the same MMSBr stock solution, the same procedure was followed for 88°, 93° and 99°C. Four tubes containing 10.5 ml buffer and 0.5 ml stock solution served as unheated controls. Also at 99°C, 0.5 ml of the MMSBr stock solution was added to each of three tubes containing 10.5 ml of M NaOH. These solutions were maintained at 99°C for 100 min to effect total decomposition of MMSBr. A previous experiment had shown 80 min at 99°C was sufficient time for total decomposition of MMSBr in M NaOH. These three tubes were also cooled in an ice-water bath. Ethylene glycol (0.11 ml) was added to all tubes and then brought to room temperature. The same procedure was used for each pH and concentration studied.

#### Quantitation of DMS production

The GLC procedure of Williams et al. (1972) was used for the quantitation of the DMS. The gas-chromatographic conditions and equipment were the same as those described by Williams et al. (1972), except that the column temperature was 80°C. The concentrations of DMS as determined by the gas chromatographic procedure were recalculated to give equivalent decomposed MMS.

#### MMS in sweet corn serum

Sweet corn serum from 'Yukon' and 'Moon-glow' hybrids was prepared by blending 500g of each with an equal weight of distilled water in a Waring Blender for 90 sec. Approximately 250 ml of the homogenates were centrifuged at 0°C for 10 min at 9,000 rpm. 100 ml of the serum supernatants were diluted with equal volumes of distilled water. The solutions were then filtered through a Whatman No. 1 filter paper.

For each hybrid, 11.5 ml of the serum were placed into each of 37 screw-cap culture tubes. One tube served as a control. Four tubes were heated in an autoclave for 45 min to completely degrade the methylnmethionine sulfonium ion and produce the total DMS potential.

Eight tubes were placed in water baths at the four temperatures. Allowing a 4 min come-up time, duplicate tubes were removed from the water baths after 20, 40, 60 and 80 min and cooled in an ice-water bath. The four tubes heated in the autoclave were also cooled in ice water. With the contents of all 37 tubes at 0–4°C, 0.11 ml ethylene glycol was added to each tube. The contents of the tubes were centrifuged for 10 min at 3,000 rpm. One phase equilibration (3 min) was made on 10 ml of each of the heated samples as previously described. A small drop of G.E. Antifoam 60 was added to the solutions just prior to removing a 10 ml sample for equilibration. The concentrations of DMS in the solutions were determined from a standard curve prepared by equilibrating 10 ml samples of standard DMS solutions prepared according to Williams et al. (1972) and recalculated to equivalent MMS.

#### Tomato cultivar serum kinetics

Two lots of whole tomato serum were prepared from 'C-28' tomatoes and an unknown tomato cultivar by extracting the juice with a tomato pulper (0.045 in. screen) and centrifuging 250 ml of the juice at 0°C for 15 min at 9,750 rpm. The C-28 serum was diluted with distilled water (1:3) to obtain a low precursor concentration and 11.5 ml quantities transferred to 36 screw-cap culture tubes. Three tubes were heated in an autoclave for 2 hr to assure total decomposition of the DMS precursor and then cooled in ice water. One unheated tube served as a control and the remaining 32 tubes were treated as described for the sweet corn serums. A 3 min come-up time was necessary for the four temperatures before reaction timing began.

Samples (11.5 ml) of the whole tomato serum of the unknown tomato cultivar were added to 33 screw-cap culture tubes. One tube served as a control and the other 32 tubes treated as previously described. To determine the total DMS potential of this serum, one part serum and three parts pH 7.0 0.2M sodium citrate buffer were combined. This permitted a reduction in heating time. Three tubes, each containing 11.5 ml of this solution, were heated in an autoclave for 45 min and then cooled in ice water.

Ethylene glycol (0.11 ml) was added to all the solutions at 0–4°C and then the solutions were brought to room temperature. One phase equilibration was made on 10 ml of each sample. The response was converted to ppm DMS from standard curves prepared according to Williams et al. (1972). The DMS concentrations were divided by .9905 to correct for the 0.11 ml ethylene glycol dilution.

The vapor pressure of the DMS standard solutions was less than the vapor pressure of DMS in the whole tomato serum or tomato

<sup>1</sup> Present address: Carnation Research Laboratories, Van Nuys, Calif.

Table 1—Rate constants ( $k \times 10^3$ ) for the degradation of MMSBr in sodium citrate buffers

MMSBr (ppm)	pH	Temperature (°C)			
		82	88	93	99
15.4	4.0	1.85	3.51	6.31	11.2
25.7	4.0	1.85	3.23	5.65	9.29
35.0	4.0	2.14	3.24	5.52	9.28
16.6	5.0	1.85	3.54	6.41	11.3
26.7	5.0	1.98	3.77	6.29	10.6
34.1	5.0	1.94	3.73	6.87	11.9
16.5	6.0	2.36	4.93	9.84	19.4
16.2	7.0	4.67	9.50	16.4	25.6
15.5	8.0	8.37	15.8	29.0	45.0

Table 2—Activation energy for the degradation of MMSBr in sodium citrate buffers

pH	MMSBr (ppm)	$E_a$ (kcal/mole)
4.0	15.4	29.8
4.0	25.7	26.8
4.0	35.0	24.5
5.0	16.6	29.9
5.0	26.7	27.6
5.0	34.1	30.1
6.0	16.5	34.8
7.0	16.2	28.1
8.0	15.5	28.1

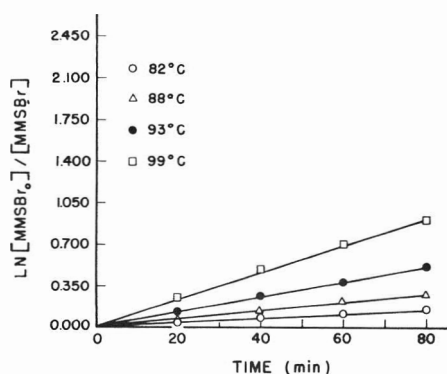
serum diluted with sodium citrate buffer (pH 7.0). This required a minor correction to standardize all solutions since only one phase equilibration was performed on each sample. The ratio of the response of DMS in the standard solutions to the response of DMS in the whole tomato serum was 0.974 while the ratio of standard solutions to the DMS response in the tomato serum diluted with pH 7.0 buffer was 0.76. After the appropriate corrections, the average DMS concentrations were determined as previously described.

## RESULTS & DISCUSSION

THE REACTION ORDER and rate of a chemical reaction depend on the concentrations of the reactants as well as the temperature, pH and the nature of the solvent. The integrated form of the rate expression for a first order reaction involving MMSBr has the form

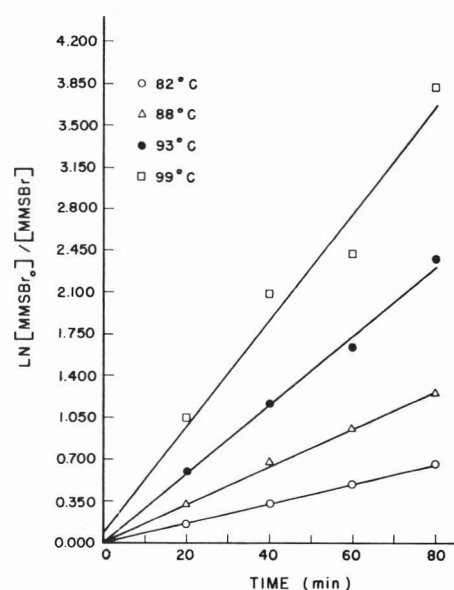
$$\ln \frac{[\text{MMSBr}_0]}{[\text{MMSBr}]} = kt$$

where  $\text{MMSBr}_0$  equals the initial concentration and  $\text{MMSBr}$  is the concentration after reaction time  $t$  (min). The plot of the natural logarithm of this ratio vs.

Fig. 1—First order kinetic plots of 15.4 ppm  $\text{MMSBr}_0$  in pH 4.0 sodium citrate buffer.

time, using linear regression, produced straight line curves for the degradation of MMSBr at pH 4.0–8.0 for each of the four temperatures studied, (Fig. 1 and 2) thus indicating first order kinetics at any pH and temperature.

The slope of the straight line curves, equal to  $k$  or the specific rate constant for the degradation reaction under the conditions specified (Table 1), showed that the rate of MMSBr degradation increased with both temperature and pH. The pH dependence suggested that the rate equation for the reaction should contain an expression for the concentration of the hydroxyl ion, but the data did not support a simple second order relationship.

Fig. 2—First order kinetic plots of 15.5 ppm  $\text{MMSBr}_0$  in pH 8.0 sodium citrate buffer.

As the pH increased the half-life value ( $t_{1/2} = 0.693/k$ ) decreased more rapidly at lower temperatures than at higher temperatures. This was evident from a plot of the half-life vs. pH for each of the four reaction temperatures at similar MMSBr concentrations (Fig. 3). The relative increase in reaction rate for an increase in temperature at constant pH can also be determined from Figure 3.

The activation energy of the reaction was determined from the Arrhenius equation under the conditions specified in Table 2. Variations in the activation energies are believed due to experimental error. The activation energy was  $27.0 \pm 2.63$  kcal at pH 4.0 and  $29.3 \pm 1.41$  kcal at pH 5.0 while at pH 7.0 and 8.0 the

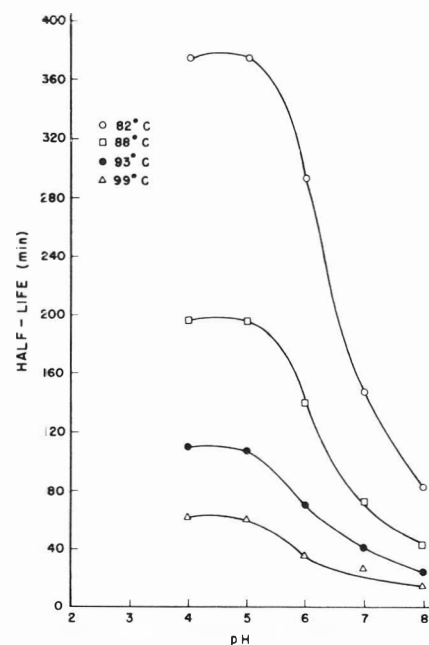


Fig. 3—Half-life of MMSBr at various temperatures and pH in sodium citrate buffer.



activation energy was 28.1 kcal. It was not possible to explain the larger activation energy at pH 6.0.

The precursor of DMS in tomatoes (Wong and Carson, 1966) and sweet corn (Bills and Keenan, 1968) has been identified as a methylmethionine sulfonium salt. The degradation of this salt in the serum of these two species should then demonstrate the same characteristics as MMSBr does in sodium citrate buffers at similar pH. The DMS precursor (calculated as MMSBr) in tomato serum and sweet corn serum exhibited first order kinetics within the temperature range studied. Figures 4 and 5 are representative first order plots. The rate constants (Table 3) for the degradation reaction in sweet corn serum were higher than for corresponding conditions in sodium citrate buffers. This indicated that the solvent composition, even though the reac-

tion remained first order, affects the specific rate constant.

The rate constants for the degradation of the DMS precursor in tomato serum (Table 3) were similar to those for MMSBr in pH 5.0 citrate buffer. The difference in the rate constants between the tomato samples might also be explained on the basis of the solvent composition. The sample containing 4.77 ppm pre-

cursor had been diluted with distilled water to obtain low precursor concentrations while the other sample was undiluted. The ionic environment may affect the reactivity of the methylmethionine sulfonium ion molecule. The concentration of the DMS precursor in tomatoes compared with the values reported by Wong and Carson (1966). They reported the level of MMS in tomato pulp, based

Table 3—Rate constants ( $k \times 10^3$ ) for the degradation of methylmethionine sulfonium salts in sweet corn and tomato serum

Product	MMSBr <sup>a</sup> (ppm)	pH	Temperature (°C)			
			82	88	93	99
Sweet corn						
Moonglow	20.8	7.0	6.90	14.8	27.6	46.6
Yukon	23.6	6.8	7.15	14.1	26.1	41.8
Tomato						
C-28 <sup>b</sup>	4.77	4.4	2.40	4.80	8.13	12.5
Unknown	38.4	4.4	2.02	3.79	6.15	11.1

<sup>a</sup> DMS precursor reported as MMSBr  
<sup>b</sup> Diluted to obtain low precursor concentration

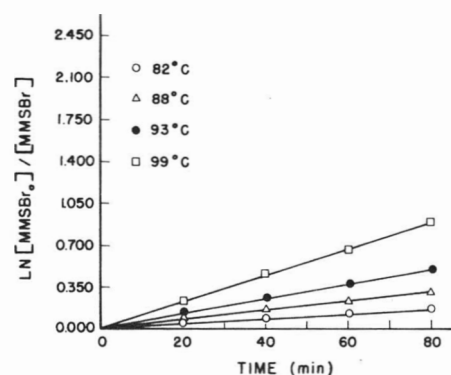


Fig. 4—First order kinetic plots of 38.4 ppm MMS ion in pH 4.4 tomato serum.

Table 4—Activation energy for the degradation of methylmethionine sulfonium salts in sweet corn and tomato serums

Product	MMSBr <sup>a</sup> (ppm)	pH	E <sub>a</sub> (kcal/mole)
Sweet corn			
Moonglow	20.8	7.0	31.6
Yukon	23.6	6.8	29.4
Tomato			
C-28 <sup>b</sup>	4.77	4.4	27.2
Unknown	38.4	4.4	27.8

<sup>a</sup> DMS precursor reported as MMSBr  
<sup>b</sup> Diluted to obtain low precursor concentration

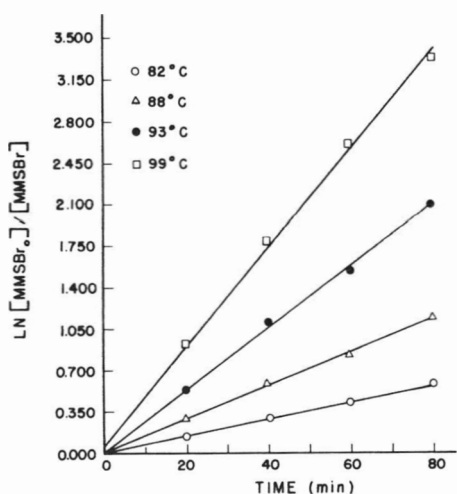


Fig. 5—First order kinetic plots of 23.6 ppm MMS ion in pH 6.3 diluted 'Yukon' sweet corn serum.

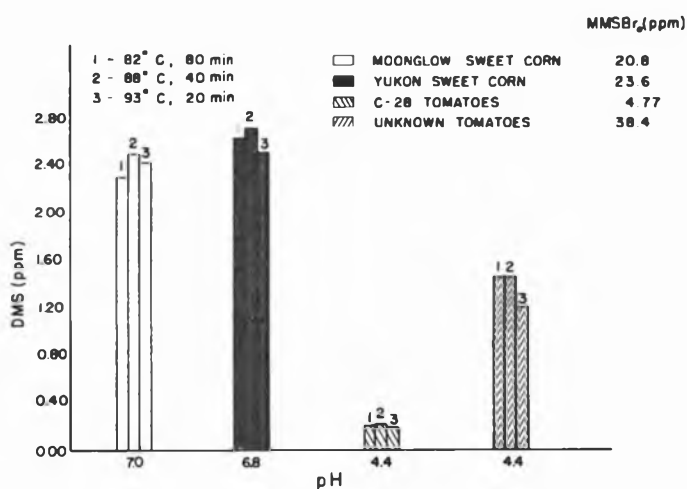


Fig. 6—Relationship of pH, time, temperature and MMS ion concentration on DMS production in sweet corn and tomato serums.

on amino acid analyses, to be from 16 to 35 ppm. The levels present in the two tomato cultivars in this study were 19.1 and 38.4 ppm.

The reaction rate (Fig. 6), measured by the production of DMS, doubled for a 5–6°C increase in temperature as did MMSBr in sodium citrate buffers. This was a very distinct characteristic of the methylmethionine sulfonium ion.

The activation energies of the reaction in either sweet corn or tomato serums (Table 4) were in good agreement with those obtained from the decomposition of MMSBr in sodium citrate buffer. The activation energies of the reaction in the two sweet corn serums were 31.6 and 29.4 kcal and in tomato serums, 27.2 and 27.8 kcal.

One may conclude from these data that the characteristics of the DMS precursor in the cultivar serums were similar to those of MMSBr in sodium citrate buffers. The precursor in all the solutions exhibited first order kinetics, a doubling

reaction rate for a 5–6°C temperature increase and similar activation energies. The three major characteristics of the decomposition of the methylmethionine sulfonium ion in sweet corn and tomato serum could, therefore, be used to identify this ion in other vegetables. The total concentration of the ion could be determined as outlined here. This method eliminates involved extraction procedures that have been used in the past to identify the methylmethionine sulfonium salts.

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## FUNCTIONAL CHARACTERISTICS OF MUSTARD MUCILAGE

### INTRODUCTION

THE MUCILAGE OF yellow mustard seed (*Brassica alba*, *B. hirta* or *Sinapis alba*) was first reported by Bailey and Norris (1932). Grant et al. (1969) reported a cold water soluble extract from mustard seeds containing cellulose in a crystalline condition. Yellow mustard seed has long been recognized by industry for its emulsification and water-binding capacity in the manufacture of prepared mustards and salad dressings. Recent efforts in our laboratory to modify the consistency of a mustard sauce with certain commercial gums resulted in an apparent incompatibility (i.e., formation of a stringy precipitate). This incompatibility led to this study in which the mucilage of yellow mustard seed was isolated, purified and its functionality characterized.

This mucilage appears to be unique to yellow mustard; it does not occur in the seed coats of brown mustard, oriental mustard (*B. juncea*) and rape seed (*B. napus* and *B. campestris*).

### EXPERIMENTAL

#### Sample material

The mustard mucilage used in this study was extracted from the hulls (bran) of yellow mustard seed. The process involved defatting of the bran with a mixture of hexane, ethanol and water. The defatted, dried bran was then water extracted (1:20 ratio) and separated using a basket centrifuge. The mucilage was precipitated from water by adding 2 vol of 95% ethanol. The mucilage was then freeze dried, followed by milling through a U.S. 80 mesh screen. Yields varied from 15 to 25% depending upon extraction conditions.

#### Rheology of mucilage

Solutions (½ to 3½%) were made using 55°C tap water in a Waring Blendor. Mixing was at low speed for 30 sec followed by high speed mixing for 2 min. The solutions were allowed to cool to room temperature and viscosities measured at various spindle speeds using a Brookfield Synchro-lectric Viscometer Model RVT.

In order to determine whether mustard mucilage possessed thixotropic, pseudoplastic or Newtonian properties, viscosities were determined on 1%, 2% and 3% solutions prepared as stated above and compared against shear rate as spindle speeds were increased, then decreased.

#### Effects of pH on viscosity

A 1% solution of mucilage was prepared by heating it on a magnetic stirrer/hot plate to 70°C and allowing it to cool to room temperature. One-half of this solution was titrated with 1N sodium hydroxide, the other half with 1N hydrochloric acid. Changes in pH and viscosity were recorded.

#### Heating and cooling cycles

A 1% solution of mustard mucilage was prepared in 10°C tap water using a Waring Blendor at low speed for 30 sec followed by high speed mixing for 1 min. The solution was then heated on a magnetic stirrer/hot plate, recording changes in viscosity every 10°C. The solution was heated to 90°C and then cooled back down to 10°C. After cooling to 10°C, the sample was frozen overnight at -15°C followed by a second heating and cooling cycle with viscosity recorded at 10°C intervals.

#### Effects of ions

Approximately 30 different salt solutions were prepared in 10% concentration to test the effects of anions and cations on the mucilage. A 1% solution of mustard mucilage was prepared by heating it to 60°C on a magnetic stirrer/hot plate. After cooling to room temperature, 1 ml of this solution was added to 25 ml of the various salt solutions. Observations were made initially and after standing overnight.

#### Chemical analysis of mucilage

The method of Mason and Slover (1971) was followed to identify the sugars in mustard mucilage. The mucilage was hydrolyzed by refluxing with a 5% solution of sulfuric acid for 4 hr. After hydrolysis, the free sugars were separated from the insoluble cellulose gel followed by neutralization with barium carbonate. The reducing sugars were reacted with hydroxylamine hydrochloride to yield their oximes. The mixture of nonreducing sugars and reducing sugar oximes was then silylated to form TMS esters which were analyzed by GLC on an SE-30 column.

The mucilage was analyzed for moisture, nitrogen, fat, reducing sugars, crude fiber, total ash and acid insoluble ash following standard AOAC procedures.

#### Surface and interfacial tensions

Mustard mucilage was compared against 17 commercial gums at levels of 0.25 and 0.50%. The hydrocolloids were prepared in one of three ways: (1) Simple rehydration in distilled water at room temperature using a magnetic stirrer; (2) Rehydration in distilled water using a magnetic stirrer/hot plate heating the solution from 25–70°C; and (3) Rehydration in distilled water using a Waring Blendor at low speed for 1 min and high speed for 6 min. Surface tension measurements were made with the Fisher Surface Tensiometer model 20 (du Nouy type).

The same procedure was followed for the interfacial tensions. However, after the platinum-iridium ring was wetted in the hydrocol-

loid solution, 25 ml of winterized soybean oil was floated on the surface of the aqueous solution. The oil and water interface was allowed to age for 60 sec before readings were taken.

#### Synergism with other hydrocolloids

In order to test the interaction of mustard mucilage with other hydrocolloids, graded series of mucilage/gum mixtures (at 0.1% intervals, totaling 1% hydrocolloid) were studied. All of the solutions in this series of experiments were made using a Waring Blendor containing 50–55°C tap water. The dry blend of the two gums to be tested was dispersed into the liquid vortex in the Waring Blendor at low speed, allowed to mix at this speed for 30 sec and then at high speed for 60 sec. The solutions were poured into jars, covered and allowed to stand overnight before viscosities were measured. This procedure generally yielded a soft gel, therefore, the solutions were shaken before viscosity readings were taken. Viscosity measurements were made using the Brookfield Synchro-lectric Viscometer Model RVT with the helipath stand using the spindles (at 5 rpm). After viscosity measurements were taken, 2 ml of concentrated acetic acid was added to each sample, lowering the pH to approximately 3.4. The samples were mixed and allowed to stand 4–5 hr before the viscosity measurements were determined. This was done because mustard mucilage was found to increase in viscosity in acidic solution.

#### Emulsification

To test the emulsifying properties of mucilage against other hydrocolloids, the following test method was developed. A vegetable oil and water emulsion was made using 49–50% water, 40% soybean oil, 10% vinegar (120 grain strength) and 0–1% of the hydrocolloid to be tested. The hydrocolloid was first dispersed in water at low speed for 60 sec in a Waring Blendor. The vinegar was then added and the slurry mixed for an additional 30 sec before the addition of the oil. After the oil had been added, the emulsion was blended at high speed for 2 min. Immediately after blending, photomicrographs were taken at 100× magnification to determine the emulsion droplet size. After the samples had cooled to 25°C, viscosity readings were taken.

Emulsion stability was determined by centrifugation (International Centrifuge Model UV). A 50-ml aliquot of the emulsion was placed in a graduated, conical centrifuge tube and centrifuged at 2000G for 14 min to break the emulsion. Emulsion stability (E.S.) was calculated as follows:

$$E.S. = \frac{\text{Emulsion volume remaining}}{\text{total initial volume}} \times 100$$

The following hydrocolloids were evaluated in both 40% and 60% oil emulsion systems: mustard mucilage, carboxymethylcellulose (type 4M6SF), propylene glycol alginate, and locust bean, guar, xanthan and tragacanth gums. Combinations of mustard mucilage and the above

<sup>1</sup> Present address: Paniplus Co., 3406 17th St., Kansas City, MO 64127

<sup>2</sup> Present address: Tragacanth Importing Corp., 141 East 44th St., New York, NY 10017

Table 1—Concentrations of mustard mucilage at various shear rates measured in centipoise at 25° C.

Spindle speed rpm	% Solution						
	0.5	1	1.5	2.0	2.5	3.0	3.5
0.5	700	7,600	20,800	40,000	62,000	100,800	138,000
1.0	550	4,400	12,200	24,000	37,000	60,000	80,000
2.5	320	2,160	5,840	11,200	18,000	29,600	40,560
5.0	210	1,280	3,280	6,200	9,600	16,400	24,000
10.0	145	760	1,860	3,550	5,400	9,100	13,240
20.0	103	470	1,060	2,063	3,050	4,960	7,200
50.0	70	253	524	970	1,470	2,344	3,360
100.0	57	159	316	592	870	1,320	1,840

Table 2—Thixotropic properties of a 1% solution of mustard mucilage as affected by increasing and decreasing shear rates at 25° C

Increasing shear, rpm	Viscosity cps	Decreasing shear, rpm	Viscosity cps
0.5	8,000	100.0	150
1.0	4,600	50.0	250
2.5	2,300	20.0	520
5.0	1,400	10.0	780
10.0	850	5.0	1,280
20.0	520	2.5	2,100
50.0	250	1.0	4,300
100.0	150	0.5	7,600

hydrocolloids at their respective maximum interactions were also tested.

Emulsions were also made using a model salad dressing formulation consisting of 40% soybean oil, 27.5% water, 10% vinegar (120 grain strength), 3% salt, 19% sugar and 0.5% hydrocolloid. These salad dressings were pre-emulsified using a Silverson Mixer-Emulsifier and then passed through a Manton-Gaulin homogenizer Model 15A (first stage pressure being 2000 psig and the second stage pressure 500 psig).

#### Suspensions

Suspensions of calcium caseinate, citrus pulp, diatomaceous earth, and liquid anti-acid preparations (aluminum and magnesium hydroxides and their silicates) were prepared in water using mustard mucilage at 0.25–1.0% as a suspending agent. The mucilage was rehydrated before addition of the other ingredients. All the samples were prepared using a Waring Blendor at low speed for 1 min. The samples were then placed in 100 ml graduated cylinders and allowed to settle. The boundary between the settled material and the supernatant liquid was recorded.

## RESULTS & DISCUSSION

### Physical and chemical properties

Mustard mucilage, a cold water-swellable gum, increased in viscosity after heating or upon standing at room temperature, to yield a low to medium viscosity solution (see Table 1). The mustard mucilage solution was opaque with a whitish-gray tint and had a bland flavor. The pH of a 1% solution of mustard mucilage varied from 6.85 to 7.25 depending upon extraction conditions.

Mustard mucilage appeared to be a linear hydrocolloid since it had thixotropic properties (Table 2), precipitated with alcohol as a stringy coagulate and showed rheological properties similar to linear gums such as tragacanth, carrageenan and guar. The rheological behavior of mucilage has been compared with organoleptic properties; mustard mucilage is a slightly slimy hydrocolloid similar to guar, tragacanth, carrageenan and karaya gums (Glicksman, 1969a).

As shown in Figure 1, the viscosity increased upon addition of either acid

(hydrochloric or acetic) or alkali, with no thinning or lowering of viscosity on standing.

Mustard mucilage is an acidic polysaccharide consisting of approximately 50% cellulose, and 10–18% uronic acids, occurring primarily as galacturonic acid (Bailey, 1935). Acid hydrolysis yields an insoluble cellulose gel and free sugars. The GLC analysis of these sugars hydrolyzed from the mucilage revealed the presence of arabinose, xylose and glucose in a 5:2:1 respective ratio. Two other unidentified saccharides were also observed.

The results of the chemical analyses are summarized in Table 3. Dialysis of an aqueous mucilage solution failed to lower the nitrogen or ash content, indicating

that neither free amino acids nor free inorganic salts were co-precipitated with the mucilage following addition of alcohol. However, if the aqueous mucilage was made acidic and dialysis carried out under acidic conditions, the ash content was significantly lowered suggesting that the mucilage polysaccharide may occur in its natural state as salts of uronic acids.

An infra-red spectrum of mustard mucilage was determined using a KBr disk. The IR spectrum obtained was identical to that reported by Krintz (1971).

The effect of a heating and cooling cycle on a 1% solution of mustard mucilage is illustrated in Table 4. The mucilage showed an increase in viscosity after a

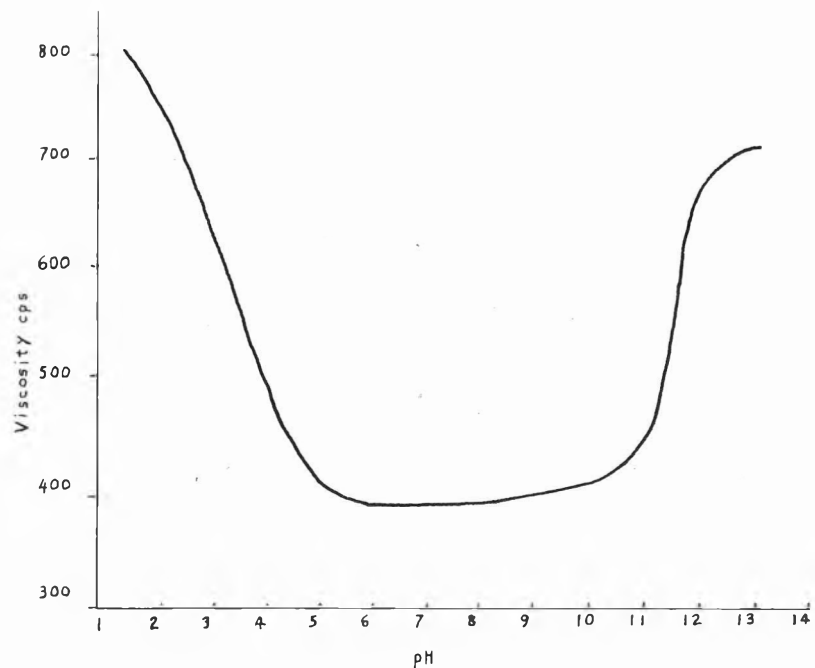


Fig. 1—pH effect on the viscosity of 1% mustard mucilage solution at 25° C measured in centipoise.

Table 3—Proximate analysis of mustard mucilage (moisture free basis)

Analysis	Mucilage from nonsolvent extracted hulls (%)	Mucilage from solvent extracted hulls (%)
Moisture %	7.75	6.69
Nitrogen %	2.30	2.38
Fat %	0.54	10.78
Reducing sugars %	1.46	0.94
Crude fiber %	33.74	33.99
Total ash %	4.55	4.77
Acid insoluble ash %	0.25	0.29

Table 4—Viscosity of a 1% mustard mucilage solution in a heating and cooling cycle

Temp °C	Viscosity cps	Temp °C	Viscosity cps
10	415	90	270
20	332	80	288
30	312	70	290
40	285	60	300
50	280	50	315
60	275	40	330
70	270	30	350
80	270	20	388
90	270	10	420

Table 5—Viscosity of a 1% mustard mucilage solution in a heating and cooling cycle after a single freeze-thaw cycle

Temp °C	Viscosity cps	Temp °C	Viscosity cps
10	350	90	248
20	325	80	255
30	308	70	268
40	285	60	278
50	273	50	290
60	265	40	327
70	259	30	339
80	252	20	350
90	248	10	385

heating-cooling cycle with little thinning at elevated temperatures. Mustard mucilage was not resistant to freeze-thaw conditions; a single cycle resulted in lower viscosity as shown in Table 5.

Solutions containing aluminum, copper, iron, lead, silver, tin and zinc ions caused mustard mucilage to precipitate from solution. Very slight precipitates

were noted on standing overnight with 10% aqueous solutions of barium, calcium and magnesium ions (as chloride salts); however, these cations as hydroxides caused precipitates to form rapidly. Slight precipitates were also noted after overnight standing with 10% aqueous solutions of phosphate, carbonate, ferricyanide, ferrocyanide, and thiosulfate ions (as

sodium or potassium salts). Precipitates or gels also form with the following test reagents for gums (Glicksman, 1969b): Millon's reagent, neutral lead acetate (20%), basic lead acetate, potassium hydroxide (10%), ferric chloride (5%), alcohol, borax (4%), tannic acid (10%) and concentrated sulfuric acid. No reaction was noted with iodine solution.

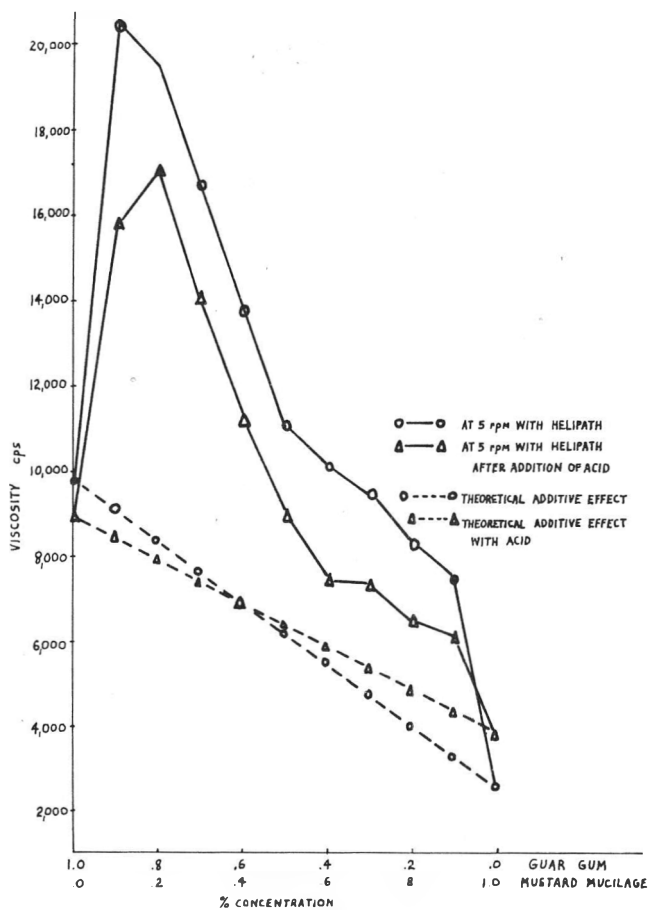


Fig. 2—Synergistic interaction of guar and mustard mucilage measured at 25° C on a Brookfield Viscometer model RVT.

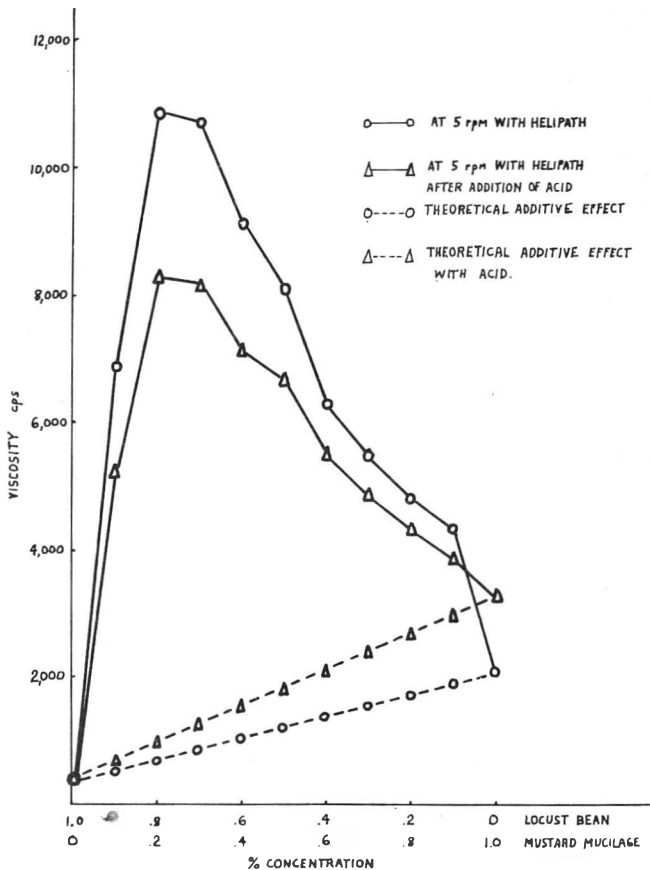


Fig. 3—Synergistic interaction of locust bean and mustard mucilage measured at 25° C on a Brookfield Viscometer model RVT.

Surface and interfacial tension

When surface tension of a mustard mucilage solution was plotted against mucilage concentration (up to 0.1%), a Type III curve (typical of surface active agents) was obtained (Becker, 1957). A Type III curve defines a rapid lowering of surface tension at low concentrations whereas a Type I curve is a gradual lowering of surface tension. A Type II curve is an increase in surface tension with increasing concentration of hydrocolloid.

Addition of mustard mucilage (0.25%) to water lowered the surface tension of water from 69.1 dynes/cm to 57.1 dynes/cm (Table 6). Three other hydrocolloids were shown to be more effective in lowering the surface tension of water, viz. 0.25% solutions of tragacanth, gelatin and propylene glycol alginate lowered the surface tension of water to 53.2, 52.7 and 46.9 dynes/cm respectively.

At the 0.5% level, mustard mucilage lowered the surface tension of water to 55.2 dynes/cm. Five other commercial gums were more effective in lowering the surface tension of water, viz. arabic, gelatin, tragacanth, ghatti and propylene

Table 6—Surface tension of hydrocolloids at 25° C in dynes/cm

	0.25% Solution	0.50% Solution
Agar <sup>b</sup>	65.9	60.0
Arabic <sup>a</sup>	65.6	53.2
CMC Type 4M6SF <sup>c</sup>	69.0	66.9
CMC Type 7M8SF <sup>c</sup>	68.6	68.6
Carrageenan, sodium <sup>b</sup>	68.8	69.2
Carrageenan, potassium <sup>b</sup>	66.3	56.1
Carrageenan, calcium <sup>b</sup>	68.8	66.9
Furcellaran <sup>b</sup>	67.9	66.3
Gelatin <sup>a</sup>	52.7	48.1
Ghatti <sup>c</sup>	63.8	45.9
Guar <sup>c</sup>	68.5	59.4
Karaya <sup>a</sup>	68.3	65.7
Locust bean <sup>b</sup>	67.8	59.9
Mustard mucilage <sup>b</sup>	57.1	55.2
Pectin <sup>c</sup>	64.2	67.3
Propylene glycol alginate <sup>c</sup>	46.9	45.8
Tragacanth <sup>c</sup>	53.2	47.7
Xanthan <sup>c</sup>	69.2	74.1
Water-air (control)	69.1	69.2

<sup>a</sup> Magnetic mixer, preparation method  
<sup>b</sup> Magnetic mixer with heating, preparation method  
<sup>c</sup> Waring Blendor, preparation method

glycol alginate lowered the surface tension to 53.2, 48.1, 47.7, 45.9 and 45.8 dynes/cm respectively.

Interfacial tension of vegetable oil and water was determined for hydrocolloids dissolved in water at levels of 0.25 and 0.50%. The data in Table 7 indicate that mustard mucilage induced the lowest interfacial tension 9.0 dynes/cm versus oil water controls of 21.5 dynes/cm followed by gelatin and tragacanth at 11.3 and 12.2 dynes/cm respectively. The ability of the mucilage to form stable emulsions appears to depend on the lowering of the interfacial tension rather than thickening.

Synergism with other hydrocolloids

Mustard mucilage reacts synergistically with guar, locust bean and carboxymethylcellulose (CMC) to produce significantly increased solution viscosity. Similar studies showing synergistic effects between xanthan gum and galactomannans have been reported recently by Kovacs (1973). For guar and mustard mucilage, the maximum synergistic effect was reached at 0.9% guar:0.1% mucilage (Fig. 2) with a viscosity of 20,400 cps (2.3× the theoretical additive value). After the additions of acetic acid to pH 3.4, this maximum shifted to 0.8% guar:0.2% mucilage with a viscosity of 17,100 cps (2.2× theoretical).

Viscosity of locust bean and mustard mucilage (Fig. 3) peaked at 0.8% locust bean:0.2% mucilage and 10,850 cps (14.5× theoretical). In the presence of acetic acid, this combination peaked at the same ratio with a viscosity of 8,300 cps (8.3× theoretical).

All commercial food grade CMC studied produced maximum viscosity when combined at the ratio 0.6% CMC and 0.4% mustard mucilage. Unlike galactomannans, guar and locust bean gum, addition of acetic acid to pH 3.4 increased viscosity of mixtures of CMC and mustard mucilage.

In comparing CMC (Hercules type 7HOF) and mustard mucilage, (Fig. 4) the maximum synergistic effect occurred at 0.6% CMC and 0.4% mustard mucilage with a viscosity of 17,700 cps (12.6× theoretical). With acetic acid addition, the same ratio prevailed, but the viscosity increased to 25,500 cps (15.9× theoretical). Given the same degree of substitution in CMC (Hercules type 4M6SF and 4H1F), the higher viscosity CMC (4H1F) showed the more dramatic synergistic increase with mustard mucilage. CMC solutions of similar viscosity (4M6SF, 300–600 cps and 7M8SF, 200–800 cps) were also studied. The less substituted CMC (4M6SF) showed a greater synergistic increase were mustard mucilage.

Smaller interactions (1.0–1.5× theoretical) were noted with tragacanth, furcellaran, xanthan and propylene glycol alginate. No interactions were noted with

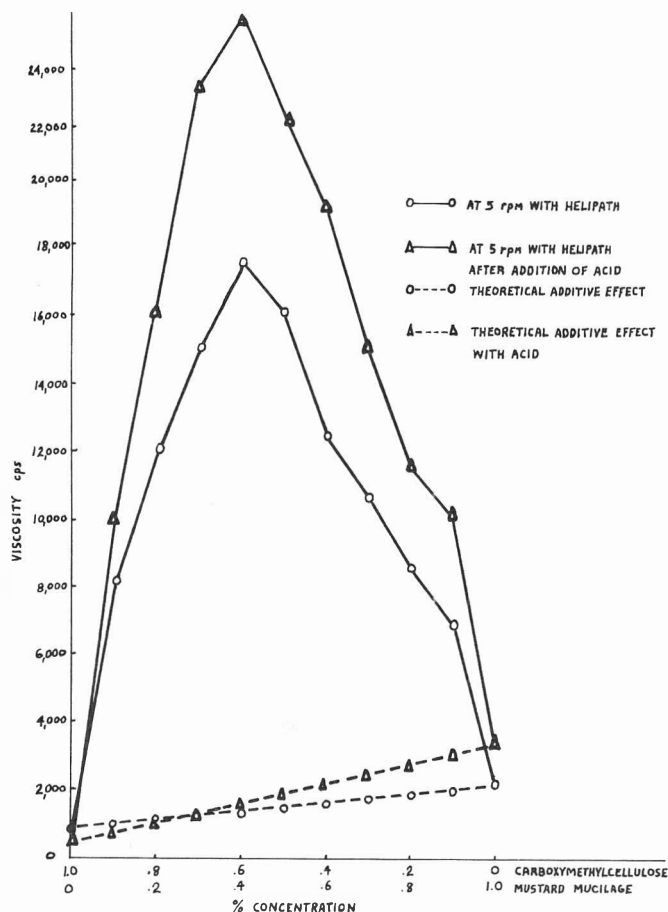


Fig. 4—Synergistic interaction of carboxymethylcellulose (Hercules type 7HOF) and mustard mucilage measured at 25° C on a Brookfield Viscometer model RVT.

the following gums: agar, arabic, sodium, potassium and calcium carrageenan, gelatin, karaya, pectin and ghatti.

#### Emulsification

Mustard mucilage acts as an emulsifier by its ability to lower the interfacial tension rather than by its contribution to build viscosity. When mustard mucilage was incorporated into a vegetable oil and water system at levels ranging from 0.25–1.00% there were corresponding increases in viscosity and emulsion stability, and corresponding decrease in oil droplet size (see Table 8). A ½% level was chosen to evaluate the other hydrocolloids since this is the common usage level of xanthan and propylene glycol alginate in salad dressings. At this concentration guar, locust bean and carboxymethylcellulose produced inferior emulsions compared to mustard mucilage. However, addition of mucilage (at maximum synergistic level) to these gums resulted in improved viscosity, emulsion stability and decreased oil droplet size. No important effect accompanied the addition of mustard mucilage to xanthan, tragacanth or propylene glycol alginate. Similar results were noted when the oil content increased from 40 to 60% in this same type of system.

With the model salad dressings made on the Manton-Gaulin homogenizer incorporating 0.5% mustard mucilage, the oil droplet size was coarser (8–10  $\mu$ ) than with xanthan or propylene glycol alginate (PGA) (3–5  $\mu$ ). Despite the particle size difference, the mucilage-containing emulsion was as stable as the xanthan or PGA-containing emulsions when subjected to freeze-thaw cycles, 38°C storage and extended storage at room temperature. When levels of 0.75–1.0% mustard mucilage were used, oil droplet sizes were similar to those obtained with xanthan or propylene glycol alginate.

#### Suspensions

Mucilage in aqueous solutions at 0.25–1.0% exhibited excellent suspending properties, which were not dependent on viscosity since samples prepared from higher viscosity hydrocolloids (e.g., CMC) settled out of solution on standing.

Calcium caseinate (10%) was suspended in water using carboxymethylcellulose (Hercules 7HOF) and mustard mucilage separately at levels of 0.25–1.0%. After 4 days 86% of a control, containing no hydrocolloid, had settled. Carboxymethylcellulose (CMC) added at levels of 0.5 and 1.0% showed significant settling after four days, 61% at the 0.5% level and 40% at the 1.0% level. Whereas the mustard mucilage tested at levels of 0.25–1.0% showed little or no settling after four days, 2% at the level of 0.25% and no settling at levels of 0.5 and 1.0%.

A similar experiment was carried out suspending 5% dehydrated citrus fruit pulp in water, using CMC and mustard

**Table 7—Interfacial tensions of vegetable oil and water as affected by hydrocolloids at 25°C in dynes/cm**

	0.25% Solution	0.50% Solution
Agar <sup>b</sup>	16.3	13.6
Arabic <sup>a</sup>	16.2	16.0
Gelatin <sup>a</sup>	11.3	10.2
Ghatti <sup>a</sup>	19.6	18.9
Karaya <sup>a</sup>	19.5	19.7
Mustard mucilage <sup>b</sup>	9.0	9.0
Pectin <sup>b</sup>	18.9	18.2
Propylene glycol alginate <sup>c</sup>	18.6	16.1
Tragacanth <sup>c</sup>	12.2	13.4
Water & oil (control)	21.6	21.4

<sup>a</sup> Magnetic mixer, preparation method

<sup>b</sup> Magnetic mixer with heat, preparation method

<sup>c</sup> Waring blender, preparation method

mucilage at levels of 0.25 to 1.0%. The control containing no hydrocolloid settled 55% after 9 days. The sample containing CMC (Hercules 7HOF) showed significant settling after 9 days, 30% at

the 0.5% level and 16% at the 1.0% level. The samples containing 0.25 and 0.5% mucilage showed little settling, allowing only a 1% separation after 9 days.

In each of the above examples viscosity data were obtained initially. The CMC samples had higher viscosities than the mucilage samples at the same concentrations.

Suspending properties of mustard mucilage were also demonstrated in several nonfood applications. A suspension containing 10% diatomaceous earth (85% less than 5  $\mu$ ) was made using 1% CMC (Hercules 4M6SF) and 1% mucilage. The control with no hydrocolloid added settled in 4 hr and the CMC sample settled in 6 days. After 2 months of storage, the mucilage sample showed no signs of settling.

A liquid anti-acid was also formulated containing aluminum and magnesium hydroxide, sorbitol, artificial sweeteners and flavors and a preservative. The control with no hydrocolloid added settled out of solution in 1 day. After 8 days, 86% of the anti-acid made with 0.75% CMC (Hercules 7HOF) had settled. After 9 months of storage the anti-acid made with 0.50% mucilage had only settled 6%.

**Table 8—Emulsification of 40% vegetable oil and water produced by hydrocolloids**

	Viscosity @ 20 rpm, cps	Droplet range $\mu$	Avg Droplet size $\mu$	Emulsion stability
0.25%				
Mustard mucilage	660	10–75	30	52.0%
0.50%				
Mustard mucilage	1810	5–50	20	82.6%
0.75%				
Mustard mucilage	3375	2–50	15	99.5%
1.00%				
Mustard mucilage	5800	2–35	8	100.0%
0.5% Locust bean	1615	15–90	45	63.5%
0.5%				
(0.8 Locust bean/ 0.2 Muc.)	2463	5–65	35	85.0%
0.5% Guar	3600	20–150	90	52.0%
0.5%				
(0.9 Guar/0.1 Muc.)	3900	10–80	50	81.0%
0.5% CMC	135	10–200	40	65.0%
0.5%				
(0.6 CMC/0.4 Muc.)	1725	5–130	20	99.5%
0.5% Xanthan	3000	5–125	8	100.0%
0.5%				
(0.8 Xan./0.2 Muc.)	2350	2–80	10	100.0%
0.5% Tragacanth	1110	10–135	45	74.5%
0.5%				
(0.7 Trag./ 0.3 Muc.)	1515	5–80	15	89.0%
0.5% Propylene glycol alginate	160	2–60	5	100.0%
0.5%				
(0.5 PGA/0.5 Muc.)	970	2–70	15	100.0%

### CONCLUSIONS

THIS PAPER demonstrates some of the properties and functional characteristics of a mucilage found in the epidermal cells that form the seed coat (hull) of yellow mustard seed. Mustard mucilage is a cold water-swellable, linear, acidic polysaccharide, containing the reducing sugars, arabinose, xylose and glucose. In aqueous solution the mucilage is a thixotropic low-to-medium viscosity hydrocolloid that exhibits surfactant, emulsification and suspending properties. The synergistic interactions with other hydrocolloids explains problems encountered in controlling the consistency of a mustard sauce with several commercial gums. Fur-

ther work is needed to elucidate more fully the chemical structure of this mucilage, and particularly the mechanism by which its cellulose content is solubilized.

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## SOME PHYSICOCHEMICAL PROPERTIES OF STARCHES AS AFFECTED BY CHANGES IN ATMOSPHERIC PRESSURE

### INTRODUCTION

BAKING AND COOKING processes are affected by atmospheric pressure (Kulas, 1950). The baker or the housewife may find that a formula, which is well balanced for sea level, produces a cake batter which rises too high in the oven and flows over the top of the pan when used at high altitude (Lorenz et al., 1971). Peterson (1930) and Lorenz and Dilsaver (1972) pointed out that as the altitude increases, the air pressure becomes less and changes in baked product formulations must be made to compensate for the difference. The formation and expansion of the leavening agents used in baking—air, steam and carbon dioxide—are of prime importance.

Starch, which is the major component of flours used in baking and cooking, also appears to be affected by changes in atmospheric pressure. Amylographs peak viscosity values of flours increase approximately 3.5% and 9.5% at elevations of 2500 ft and 5000 ft, respectively, over those obtained at sea level conditions (Lorenz, 1973a). The operation of the mixograph is influenced by changes in atmospheric pressure. Maximum curve height, the time to maximum curve height, and the area under the curve increase as elevation increases (Lorenz, 1973b).

Since approximately one-third of the United States lies in the high-altitude region, a study of the effects of varying atmospheric pressures on the physicochemical properties of starches such as water-binding capacity, viscosity and surface area particle size was undertaken.

### MATERIALS & METHODS

#### Starch samples

Commercial potato, rice, corn and wheat starches were used for the experiments. Average surface area particle size determinations of each starch were made with a Fischer Sub-Sieve-Sizer.

#### High altitude chamber

All studies were carried out in a special laboratory which consists of a steel cylinder seven feet in diameter and nine feet in height. This laboratory can be ventilated and temperature controlled. The atmospheric pressure inside this laboratory can be adjusted and maintained between 770 and 500 mm Hg.

#### Photomicroscopy

Photomicrographs of the four starches were taken with a Leitz Orthomat-Ortholux photomicroscope at five temperatures: 22°, 60°, 70°, 80° and 90°C. The magnification was 400× for the corn, wheat and rice starches and 100× for the potato starch. The smaller magnification for the potato starch was necessary because of the

large swelling of the potato starch. This swelling made it difficult to photograph potato starch granules at a larger magnification, particularly at the higher temperatures. The highest temperature, 90°C, was chosen because at 10,000 ft a higher temperature cannot be attained for dilute starch suspensions.

The starch suspensions were all 1% concen-

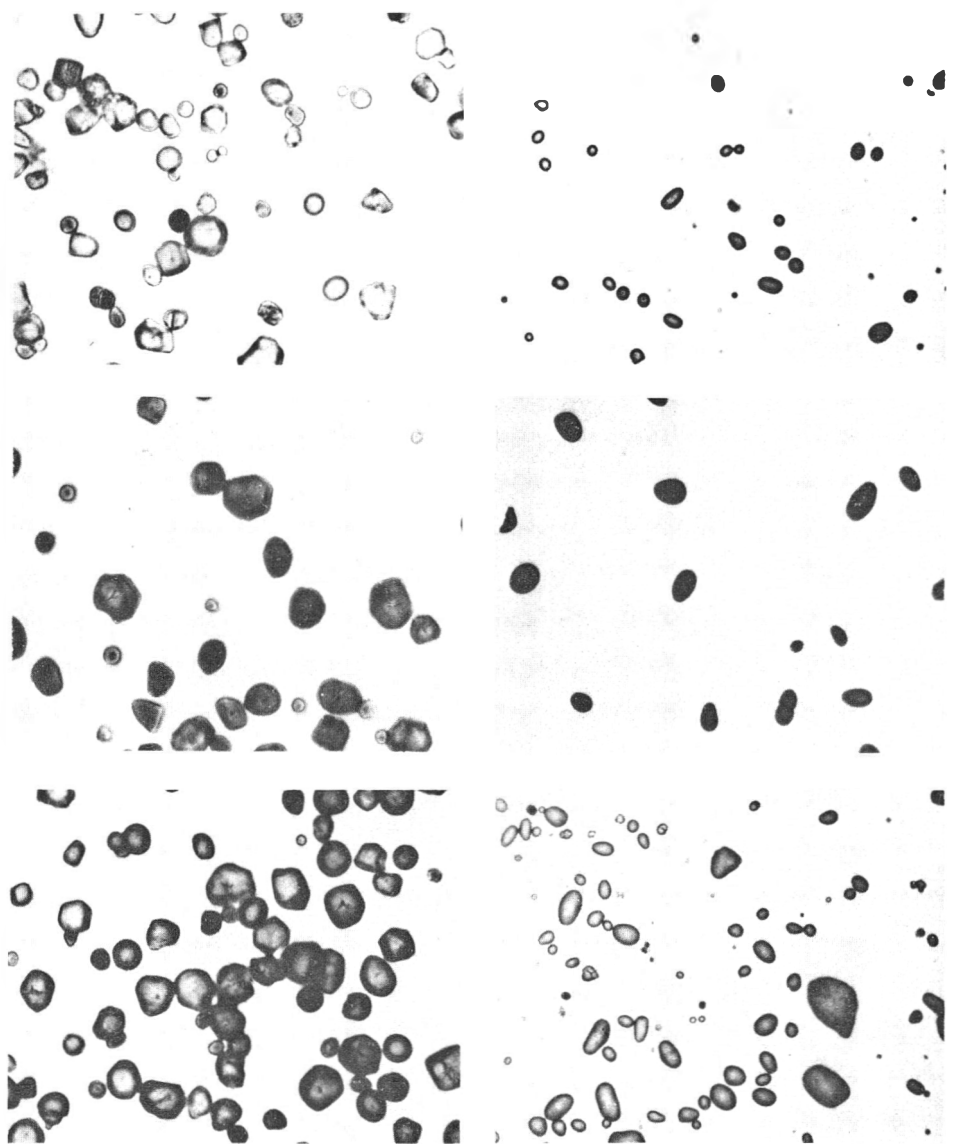


Fig. 1—Photomicrographs of corn (left) and potato (right) starch granules at sea level (upper), 760 mm Hg; 5000 ft (center), 640 mm Hg; and 10000 ft (lower), 520 mm Hg.

trations in water. The five altitudes were sea level, 2500, 5000, 7500 and 10,000 ft.

A sample of the starch suspensions at 22°C was transferred from the beaker to a glass slide, stained with a 0.1% aqueous iodine solution, covered, placed on the microscope stage and photographed. The starch suspensions then were heated to 60°, 70°, 80° and 90°C successively, a sample transferred to a slide, stained and photographed. This procedure was followed at each of the five altitudes.

Slides of the photomicrographs of all four starches at 22°C were projected to 100 times the diameter of the slides and starch granule size was measured manually with a centimeter rule. This was a procedure adapted from Schoch and Maywald (1959). These measurements were used to compute the average starch granule surface area at 5000 and 10,000 ft of elevation, based on the actual size determined at sea level with the Fischer Sub-Sieve-Sizer. The data were analyzed statistically.

#### Water-binding capacity

The procedure followed was the method reported by Medcalf and Gilles (1965) with modification. Starch water-binding capacity was measured by adding 0.5g of starch to 10 ml of distilled water in a tared 15 ml centrifuge tube. The tubes were stoppered and agitated for 1 hr with a Burrel shaker. This was followed by centrifuging for 10 min at 5000 rpm using an International Equipment Co. centrifuge. Excess water was allowed to drain off and the tube was weighed and the bound water determined. The formula,  $[g \text{ bound water} \times (100/5)]$  as used by Medcalf and Gilles (1965), was used to calculate the percent of water-binding capacity. Three determinations were made at each of the five altitudes. The results were subjected to a statistical analysis.

#### Viscosity measurements

Viscosity measurements were made with the Brookfield Synchro-Lectric Viscometer. Concentrations of the starch suspensions were: corn—3%; potato—0.5%; rice—3%; wheat—6%. These concentrations were determined in preliminary experiments in order to obtain a reading at the low speed (20 rpm) and avoid a very high reading at the high speed (50 rpm), especially at post-gelatinization temperatures. The use of a magnetic stirrer prevented the settling of starch granules at pregelatinization temperatures.

The viscosity of the starch suspensions was measured by taking the initial readings at 22°C. The suspensions were then heated to 40°, 60°, 80° and 90°C, respectively, and readings made at these temperatures.

It was thought that the total solids concentration might be affected by the evaporation of water from the starch solutions particularly at the higher elevations. This evaporation, if great enough, could influence viscosity at these elevations. Before each reading of viscosity, the suspensions were weighed in preliminary experiments to measure moisture loss. At 10,000 ft of elevation the evaporation loss ranged from 3.2–4.7% depending on the kind of starch. This moisture lost due to evaporation at higher elevations was replaced before viscosity readings (reported in Table 3) were made. The viscosity of each starch suspension was measured at each of the five altitudes. All experiments were conducted in duplicate. The data were analyzed statistically.

## RESULTS & DISCUSSION

### Photomicroscopy

Visual observation of the rice, corn and potato starch granules in suspension at 22°C showed that the surface area particle size at sea level was smaller than at 5000 ft and 10,000 ft. Wheat starch did not show such a surface area particle size difference. The difference in particle size of corn and potato starch granules with increasing elevation is illustrated in Figure 1.

At 70°C all four starches were past their gelatinization ranges. It was impossible to measure the effects of altitude quantitatively on these pictures, but qualitatively, some differences could be noted. Photomicrographs at this temperature showed that granules of corn and wheat starches were enlarged and irregular at each elevation. The granules of both starches appeared intact and the characteristic concaveness of wheat starch granules was still apparent.

Rice starch clearly showed the effects of heat and altitude. At sea level and 70°C, the granules were swollen but intact, while at 10,000 ft of elevation the granules seemed to have ruptured. Potato starch showed little effect of altitude at this temperature. The granules were in

various stages of swelling. At 90°C the photomicrographs of all starches indicated ruptured starch granules when the experiments were carried out at 10,000 ft of elevation.

The surface area particle sizes of starches in dilute suspensions measured by projecting the slides of photomicrographs by the procedure of Schoch and Maywald (1959) are given in Table 1. Each value is the average of the diameters of the ten smallest granules and the ten largest granules. Corn, potato, and rice starches showed an increase in surface area granule size with increases in elevation. Wheat starch did not.

To determine the significance of the measurements in Table 1, an analysis of variance (AOV) was conducted. The small and large granules of corn starch showed a significant increase ( $\alpha = 0.05$ ) in size on changing elevation from sea level to 5000 ft. Changing elevation from 5000 ft to 10,000 ft the increase was insignificant. A significant increase in surface area particle size of the potato starch was only observed for the large granules.

Rice starch paralleled corn starch in that both the small and large granules significantly ( $\alpha = 0.05$ ) increased in size as the altitude increased from sea level to 5000 ft. There was no significant increase

Table 1—Surface area particle size comparisons (22°C) of starches at different elevations

Starch	Fischer sub-sieve-sizer ( $\mu$ )	Altitude (ft)	Projected granule size ( $\mu$ )	
			Small	Large
Corn	13.9	0	7.1	20.7
		5000	9.4	24.5
		10000	9.6	25.9
Potato	25.0	0	9.8	40.2
		5000	9.8	60.0
		10000	9.2	76.5
Rice	10.4	0	4.8	16.0
		5000	6.2	19.0
		10000	6.0	19.5
Wheat	20.3	0	5.3	35.3
		5000	4.9	34.8
		10000	5.5	35.5

Table 2—Average water-binding capacity  $[g \text{ bound water} \times (100/5)] = \% \text{ bound water}$  <sup>a</sup> of starches at different elevations

Starch	Elevation (ft)					F Values
	0	2500	5000	7500	10000	
Corn	81	83	84	86	95	10.6*
Potato	80	83	92	97	96	5.2*
Rice	86	88	84	86	86	1.0
Wheat	92	93	88	92	90	0.5

<sup>a</sup> Medcalf and Gilles (1965)

\* ( $\alpha = 0.05$ )

in size for rice starch granules above 5000 ft. The size of wheat starch granules was not significantly affected by increases in elevation.

**Water-binding capacity**

The average water-binding capacity for each starch at the different altitudes is given in Table 2. The values obtained for the four starches at sea level were in agreement with the findings of other researchers (Kulp, 1972; Schoch and Maywald, 1959).

The values for corn and potato starches increased as the altitude increased. Rice and wheat starches showed no effect of altitude, remaining relatively constant.

To analyze the effect of altitude, a one-way AOV was used. The F values as given in Table 2 showed that water-binding capacities of corn and potato starches are affected by altitude ( $\alpha = 0.05$ ). This significance correlated with the increased size of the corn and potato starch granules as seen in the photomicrographs (Fig. 1) of starch granules at sea level, 5000 and 10,000 ft.

**Viscosity measurements**

Viscosity measurements made on four starch suspensions at five altitudes with a Brookfield Viscometer and recorded at five specific temperatures are given in Table 3.

The viscosities of corn and rice starch decreased with an increase in temperature between 22° and 60°C for elevations from sea level to 7500 ft. For potato starch and wheat starch, this decrease in viscosity was recorded only between 22° and 40°C.

The gelatinization range of potato starch is 50–69°C; that of wheat starch is 52–64°C (Whistler and Paschall, 1965). Corn starch and rice starch, however, never begin gelatinization until past 60°C.

The viscosity of water decreases with increasing temperatures (Bingham and Jackson, 1918). At 22° and 40°C, suspensions of potato starch and wheat starch are still essentially water systems, so it would be expected that the viscosities at these temperatures would decrease. At 60°C an increase in viscosity would be expected. Rice and corn starch viscosities should decrease even at 60°C. Once gelatinization begins, the viscosity of all starch suspension would be expected to increase and this is shown in Table 3.

With the exception of potato starch, viscosities decreased with an increase in elevation from sea level to 7500 ft. Decreases in atmospheric pressure allow the granules, swollen and unswollen, to move more freely, therefore reducing resistance to flow at higher elevations. Thus, viscosity is reduced at increased elevations. This would explain the initial decrease in

viscosity from sea level to 7500 ft as seen in Table 3. However, the countereffect of gelatinization at 80° and 90° would cause an increase in viscosity even at the higher elevation. Thus, there is a final rise in cps at 10,000 ft.

The four temperatures from 22° to 80°C did not show as great a fluctuation in viscosity due to altitudes as 90°C has shown. At 60°C (Fig. 2) potato and wheat starches increased in initial viscosity measured at 20 rpm over that recorded at 22°C showing the effects of initial gelatinization. Corn and rice starches still showed decreased viscosities.

Figure 3 shows the viscosities at 90°C. Corn and wheat starches followed a similar pattern. Sea level, 2500, 5000 and 7500 ft gave decreasing viscosity readings at 90°C; 10,000 ft showed a higher reading, falling at a point between those for sea level and 2500 ft in the case of corn starch, and between 2500 ft and 5000 ft in the case of wheat starch.

The final rise of viscosity for suspensions of wheat, rice and corn starches is evident. Potato starch showed no effect.

The effect of altitude on the viscosity of starch suspensions was analysed by a two-way AOV. The effect of altitude was significant ( $\alpha = 0.05$ ) with all starches except potato. As expected the effect of temperature was also significant for all starches. The interaction of altitude and

Table 3—Average viscosity data for all starches at 20 and 50 rpm (cps)

Altitude (ft)	C°	Corn starch (3%) average cps		Potato starch (0.5%) average cps		Rice starch (3%) average cps		Wheat starch (6%) average cps	
		20 rpm	50 rpm	20 rpm	50 rpm	20 rpm	50 rpm	20 rpm	50 rpm
		0	22	4.0	7.8	3.5	7.1	3.75	7.8
2500	22	3.75	7.6	3.5	7.4	3.75	7.6	4.0	7.9
5000	22	3.5	6.9	3.75	7.2	3.5	7.5	4.0	8.0
7500	22	3.5	7.4	3.75	7.0	3.5	7.9	4.0	8.3
10000	22	2.75	7.3	3.5	7.3	3.0	7.45	3.5	8.2
0	40	3.75	6.3	3.5	6.4	3.0	6.75	3.75	7.1
2500	40	3.5	5.7	3.25	6.5	3.25	6.4	3.5	5.8
5000	40	3.0	5.6	3.0	5.9	2.5	6.0	3.0	5.8
7500	40	2.5	5.7	3.25	5.8	2.25	5.7	3.5	5.7
10000	40	3.0	6.5	2.75	6.7	2.75	6.35	3.25	6.9
0	60	3.25	6.2	5.0	9.0	2.5	5.4	8.75	12.1
2500	60	3.25	5.7	4.25	8.2	2.75	5.1	8.5	12.1
5000	60	3.0	5.6	4.0	7.7	2.5	5.3	8.0	11.7
7500	60	2.5	5.5	4.0	7.4	2.5	5.4	7.5	10.9
10000	60	3.25	7.3	5.0	8.1	2.5	5.2	7.25	12.6
0	80	8.0	14.2	10.5	16.6	5.0	9.1	24.5	27.1
2500	80	7.5	13.5	8.0	15.1	4.75	8.6	18.0	22.6
5000	80	6.0	10.2	7.5	13.9	4.25	8.8	13.0	20.7
7500	80	5.0	10.4	9.5	16.4	4.5	8.9	9.75	15.8
10000	80	5.5	10.6	9.5	15.7	5.0	8.8	10.5	16.3
0	90	64.0	52.0	50.0	42.4	13.5	26.0	228.0	160.4
2500	90	33.0	41.6	54.0	45.0	10.0	21.6	114.0	86.4
5000	90	27.0	37.6	51.0	43.2	10.5	18.8	81.0	72.8
7500	90	23.0	37.2	53.0	47.2	8.0	15.2	61.0	62.0
10000	90	38.0	43.2	46.0	50.8	25.0	29.6	94.0	97.2

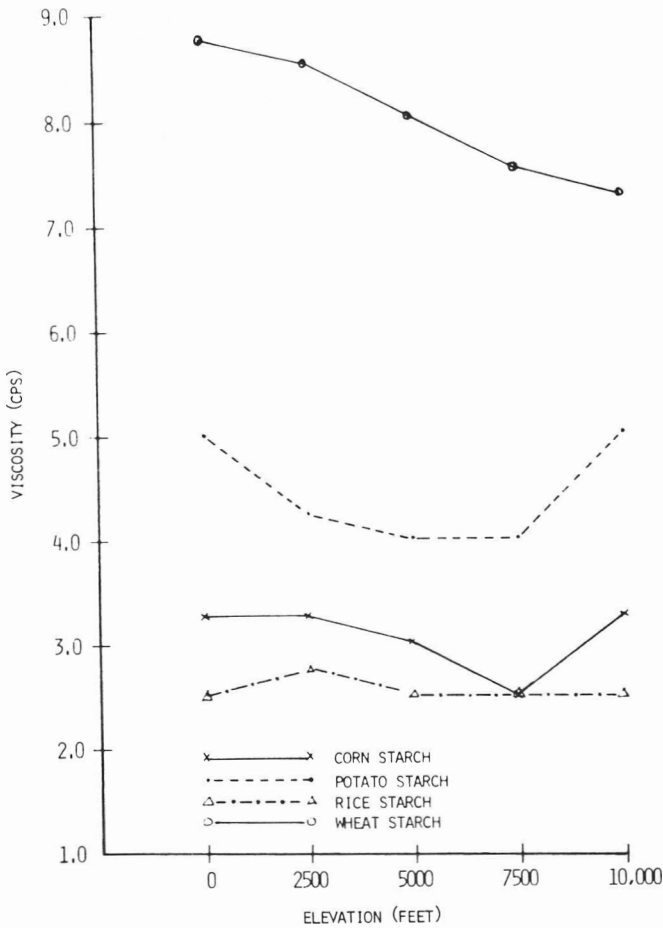


Fig. 2—The effect of elevation on starch viscosity at 60° C.

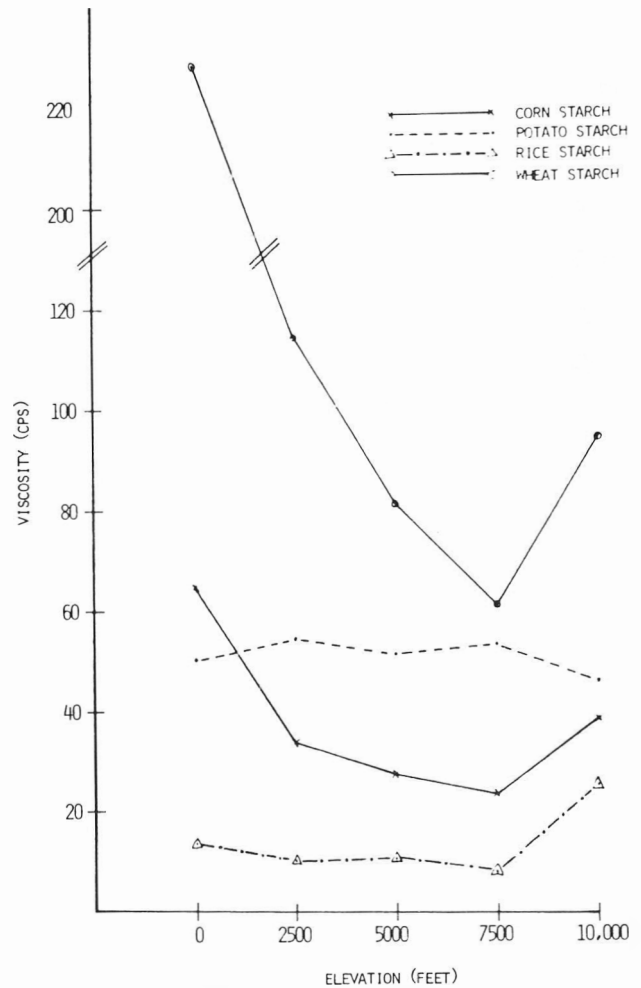


Fig. 3—The effect of elevation on starch viscosity at 90° C.

temperature was significant for all starches except the potato starch.

CONCLUSIONS

MEASUREMENTS of the photomicrographs indicated that corn, potato and rice starch granules in dilute suspensions increased in surface area particle size with increases in elevations. The increase in size of corn and potato starch granules was correlated with increased water-binding capacity of these starches at greater elevations. Both corn and potato starches showed a significant increase in water-binding capacity as the altitude increased, while the values for rice and wheat starches were not significant. The viscosity of corn, rice and wheat starch suspensions were significantly affected by increases in elevation. The viscosity decreased with initial increases in elevation at pregelatinization temperatures due to

less stress on the system at lower levels of atmospheric pressure. At lower temperatures, the suspensions reflected the viscosity of water, decreasing with increases in temperature. Suspensions heated to gelatinization temperatures showed an increase in viscosity. This increase occurred at a lower temperature for potato and wheat starches because they begin to gelatinize at a lower temperature than corn or rice starches. At higher temperatures, the gelatinization of the starches was sufficient to counter the effect of altitude, thus increasing viscosity at the highest elevation.

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## CEREBROSIDE IN RICE GRAIN

### INTRODUCTION

RECENTLY, Fujino and Sakata (1972) noted that in rice grain there are at least three groups of glycolipid, i.e., cerebroside (hexosylceramide), glyceroglycolipid and sterol glycoside. The present paper is concerned with the isolation of cerebroside from rice grain and with chemical characterization of the lipid.

### EXPERIMENTAL

#### Isolation of rice grain cerebroside

**Preparation of crude glycolipid.** 3 kg of rice grain were milled and extracted with chloroform-methanol (2:1, v/v). The extract was washed with water, dehydrated with sodium sulfate and condensed under reduced pressure to yield about 79g of total lipid. This was then applied to silicic acid column chromatography by sequential elution with chloroform, acetone and methanol (Rouser et al., 1967). Crude neutral lipid was eluted with chloroform, crude glycolipid with acetone and crude phospholipid with methanol. 7.7g of crude glycolipid were thus prepared.

**Purification of cerebroside.** The crude glycolipid was subjected to silicic acid column chromatography with a mixture of chloroform and acetone (Vorbeck and Marinetti, 1965). The fractions eluted with chloroform-acetone (1:1 to 1:4, v/v) were combined and condensed to obtain cerebroside, which was purified by treatment with mild alkali and by re-chromatography on silicic acid column with chloroform-acetone. The yield of purified cerebroside was 85 mg. The lipid was identified by silica gel G thin-layer chromatography (TLC) and infrared (IR) spectrometry.

#### Hydrolysis of rice grain cerebroside

**Preparation of fatty acid methyl esters and methyl glycosides.** 8 mg of rice cerebroside were refluxed for 6 hr with 5% HCl in methanol. After hydrolysis, the mixture was cooled and extracted with hexane. The extract was washed with water, dehydrated and condensed under reduced pressure to produce methyl esters of the component fatty acids. A part of the esters was fractionated into normal and hydroxy fatty acid methyl esters on silica gel G plate developed with hexane-ether (85:15, v/v). The residual mixture, having been extracted with hexane, was taken to ion-exchange column chromatography with Amberlite IR-4B (OH<sup>-</sup>). The constituent sugars were obtained from the effluent as methyl glycosides by condensation under reduced pressure.

**Preparation of long-chain bases.** 10 mg of rice cerebroside were hydrolyzed for 18 hr at 70°C with 1N HCl in water-containing methanol (Gaver and Sweeley, 1965); the mixture was cooled and extracted with hexane. Long-

chain bases were subsequently extracted at pH 10 from the residual solution and purified by sequential elution with chloroform and methanol on silicic acid column.

#### Gas-liquid chromatographic analysis

Constituents of rice cerebroside were assayed by using GLC. The apparatus, a Hitachi 063 chromatograph (Hitachi Seisakusho Co. Ltd., Tokyo), was equipped with a hydrogen flame ionization detector. The normal fatty

acid methyl esters were analyzed at 175°C on a glass column (0.3 × 200 cm) packed with 10% DEGS. The hydroxy fatty acid methyl esters and long-chain bases were trimethylsilylated and analyzed at 210°C on the glass column packed with 5% SE-30. The methylglycosides were trimethylsilylated and analyzed at 175°C on the glass column packed with 5% SE-30. The carrier gas in all analyses was N<sub>2</sub>.

### RESULTS

#### Identification of rice grain cerebroside

Rice cerebroside was put on a thin-layer borax-impregnated silica gel G plate, developed with chloroform-methanol-water (24:7:1, v/v/v) and located by spraying with anthrone reagent. Only a single spot was revealed on the plate and the R<sub>f</sub> value agreed well with that of a glucosylceramide isolated from bovine milk (Fujino et al., 1969), but not with that of a galactosylceramide.

Infrared spectrum of rice cerebroside (Fig. 1) gave peaks at 3440 cm<sup>-1</sup> and 1125 ~ 1000 cm<sup>-1</sup> for hydroxy groups of sugar moiety; peaks at 1640 cm<sup>-1</sup> and 1550 cm<sup>-1</sup> for acidamide linkage; a peak at about 900 cm<sup>-1</sup> for β-glucopyranosidic bond; and a peak at 970 cm<sup>-1</sup> for trans double bond, but no peak at 1735 cm<sup>-1</sup>

Table 1—Fatty acid composition of rice grain cerebroside

Fatty acid	%
14:0	0.6
16:0	0.7
18:0	0.8
18:1	0.2
18:2	0.2
20:0	1.0
22:0	0.2
24:0	0.3
18h:0 <sup>a</sup>	7.2
20h:0	59.0
22h:0	10.3
24h:0	19.5

<sup>a</sup> h means 2-hydroxy.

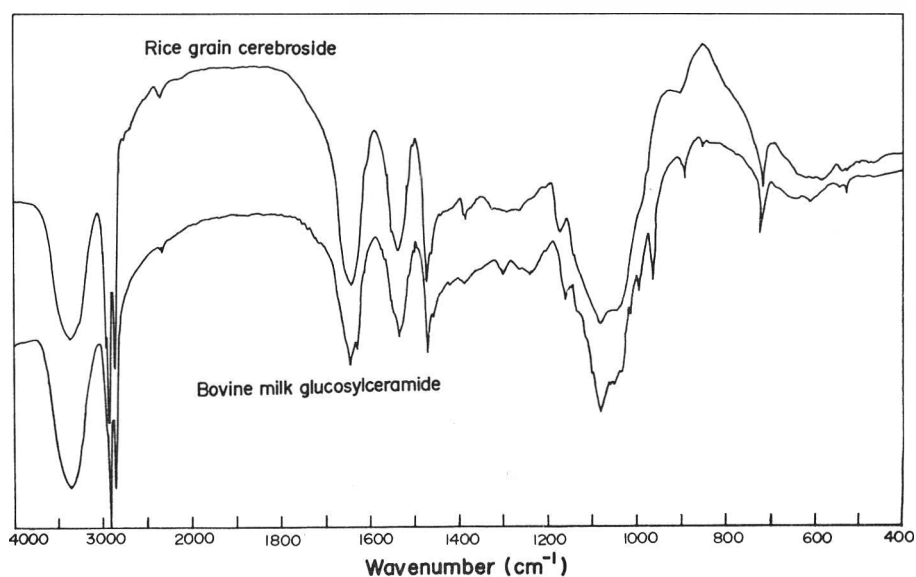


Fig. 1—Infrared spectrum (in KBr) of rice grain cerebroside.

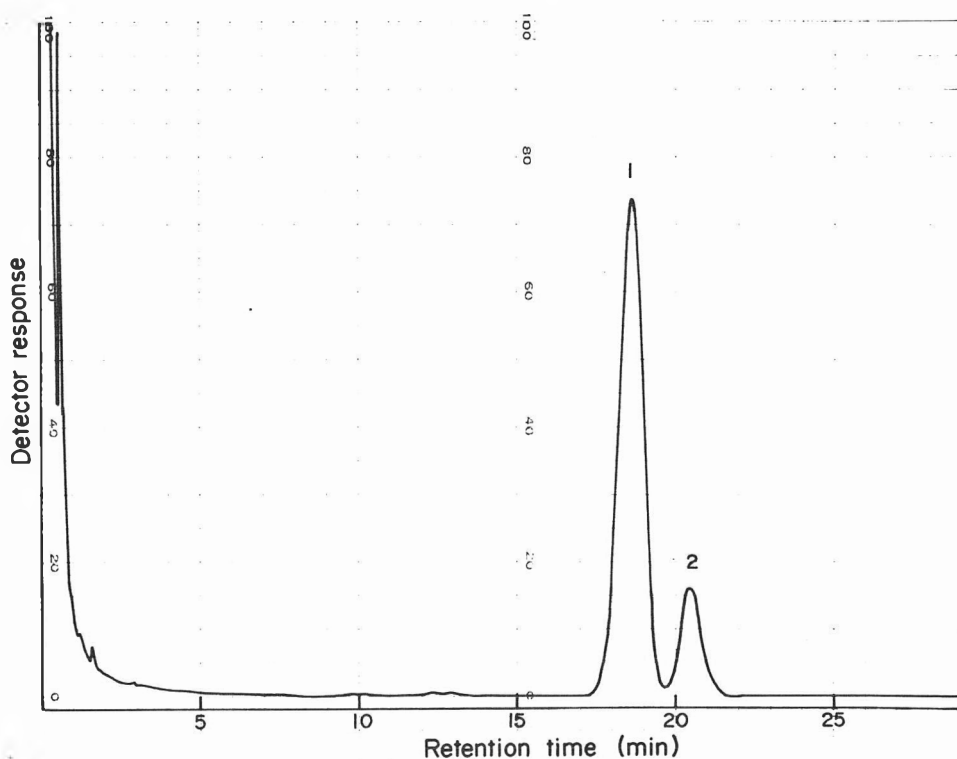


Fig. 2—Gas-liquid chromatogram of sugar in rice grain cerebroside: (1)  $\alpha$ -glucose; (2)  $\beta$ -glucose.

for ester linkage. The IR pattern coincided with that of the glucosylceramide standard (Fujino et al., 1969).

#### Component fatty acids in rice grain cerebroside

The ratio of hydroxy and normal fatty acid methyl esters was developed densitometrically 96:4 on silica gel G plate with hexane-ether (85:15, v/v). Composition of the fatty acids calculated from the ratio and peak areas in the GLC charts for hydroxy and normal fatty acid methyl esters is shown in Table 1. 12 peaks found in the charts were identified by comparison of the relative retention times with those for the known fatty acid methyl esters (Nakano et al., 1972). Major acids included 2-hydroxyarachidic, 2-hydroxylignoceric (= phrenosinic), 2-hydroxybehenic and 2-hydroxy stearic acid, among which the first one occupied more than half of all fatty acids in the rice cerebroside.

#### Component sugar in rice grain cerebroside

A GLC chart for the component sugar in rice cerebroside is shown in Figure 2. The peaks were identified by co-chromatography with a standard preparation of trimethylsilyl ether derivatives of methylhexosides. As seen, two peaks implicating  $\beta$ -glucose and the  $\alpha$ -anomer were revealed, but no other peaks. This signifies that the sugar component in rice cerebroside is glucose.

#### Component long-chain bases in rice grain cerebroside

A GLC profile for the component long-chain bases in rice cerebroside is depicted in Figure 3. An attempt was made to identify the peaks by co-chromatography with a standard preparation of trimethylsilyl ether derivative of sphingosine ( $C_{18}$ ) and by comparison of the relative retention times with those in the literature (Carter and Gaver, 1967). Three bases were recognized: sphingosine ( $C_{18}$ ), dehydrophytosphingosine, and an unidentified base, among which sphingosine (72.9%) was dominating.

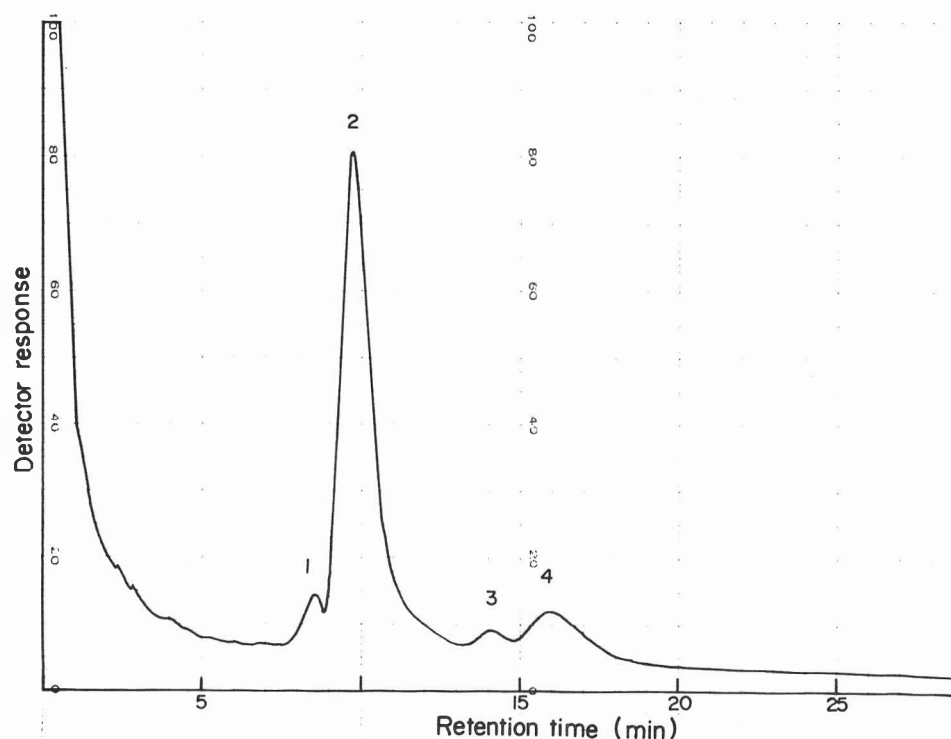


Fig. 3—Gas-liquid chromatogram of long-chain bases in rice grain cerebroside: (1) 3-O-methyl- $C_{18}$ -sphingosine (Artificial, 5.1%); (2)  $C_{18}$ -sphingosine (72.9%); (3) unidentified (4.7%); (4) dehydrophytosphingosine (17.3%).

## DISCUSSION

THE DATA SHOW that rice grain cerebroside is chemically composed of 12 fatty acids (2-hydroxyarachidic being predominant), one sugar (glucose), and three long-chain bases a greater part of which is sphingosine. Based on the results, together with the fundamental chemical structure of cerebroside (Carter et al., 1956), the major species of the rice grain cerebroside would be characterized as N-2-hydroxyarachidyl- $\beta$ -D-glucopyranosyl-(1-1')-sphingosine.

Cerebrosides have been less investigated so far in the field of plants and microorganisms than in the animal king-

dom. Regarding the plant field, as far as we know, cerebrosides have been studied in detail only on wheat flour (Carter et al., 1961), runner bean leaves (Sastry and Kates, 1964) and bush bean leaves (Carter and Koob, 1969). Our work demonstrates that cerebroside exists in rice grain as well. Plant cerebrosides as reported in the literature commonly contain 2-hydroxy fatty acids as the chief component acid, only glucose as the constituent sugar and a phytosphingosine analogue as the main component long-chain base. In the rice grain cerebroside, the major component acids and the sole constituent sugar were 2-hydroxy acids and glucose, respectively; however, the principal long-chain base

was not a phytosphingosine analogue but sphingosine.

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## FORMATION OF N-NITROSAMINES IN LOW MOISTURE SYSTEMS

### INTRODUCTION

N-NITROSAMINES have been reported to occur in nitrite-treated herring meal (Sakshaug et al., 1965; Sen et al., 1972), meat products (Ender and Ceh, 1968; Sen, 1972), wheat flour (Kröller, 1967; Marquardt and Hedler, 1966), cheese (Marquardt and Hedler, 1966), African alcoholic beverage (McGlashan et al., 1968) and a South African Bantu food plant (Duplessis et al., 1969). However, as pointed out by Pensabene et al. (1972) and Sen (1972), the accuracy of some of these reports is doubtful as many of the techniques used in these studies are not completely definite. It follows that if the methods themselves are suspect, the results obtained are also suspect as a number of other food components are known to appear as artifacts in the analyses. Devik (1967), for example, using a polarographic detection system, reported the formation of N-nitrosamines on heating a mixture of D-glucose and L-alanine but later publications confirmed these compounds as methyl substituted pyrazines (Fujimaki et al., 1972; Havre and Ender, 1971; Scanlan and Libbey, 1971).

It has also been shown by Sen et al. (1969) that secondary amines and nitrite can form N-nitrosamines when incubated with human gastric juice. Secondary amines have been reported in a number of foods including fish (Gruger, 1972; Wick et al., 1967), vegetables (Preusser, 1966) and fruit juices (Stewart et al., 1964). Large concentrations of nitrite have been found in stored green vegetables especially spinach, celery and green salad as a result of bacterial reduction of nitrates (Sinios and Wodsak, 1965). Nitrites and nitrates are also widely used as preservatives and color fixatives in fish and meat products as prescribed in the United States Food Additive Regulations, 121.1063 and 121.1064.

A limited amount of research has been reported on the formation of N-nitrosamines from secondary amines and sodium nitrite in model systems. Ender and Ceh (1971) discussed N-nitrosamine formation in model experiments when sodium nitrite in great quantities was brought into reaction with alkylamines, amino acids and proteins from meat and fresh water fish. They concluded that the formation of large quantities of N-nitrosamines in biological systems depended on the presence of nitrite and the actual

amine, pH, temperature and time of reaction and also on the presence of a protecting colloid such as starch or glutinous substances which prevent the evaporation of the formed N-nitrosamines at high temperatures.

In this paper, factors which could influence the amount of N-nitrosamine formed within a low moisture content freeze-dried carboxymethylcellulose system were investigated. It is expected that this system will be a forerunner to more complicated food systems containing protein and lipid.

### EXPERIMENTAL

#### Materials

Dimethylnitrosamine (DMN), diethylnitrosamine (DEN), dipropylnitrosamine (DPN), dibutylnitrosamine (DBN), dimethylamine hydrochloride, diethylamine, dipropylamine and dibutylamine were obtained from Eastman Organic Chemicals, sodium nitrite and diethyl ether from Baker Chemical Company. The latter was purified by the method of Croteau and Fagerson (1968) before use. The matrix used for the model system was cellulose gum (CMC), type 7HF (Hercules Inc.).

#### Preparation of freeze-dried model systems

The reaction between sodium nitrite and secondary amine was studied in model systems simulating freeze-dried or other dried foods. An inert matrix consisted of a cellulose emulsion prepared according to the method of Bishov et al. (1960). Cellulose gum (2.5g) was dispersed in a borate buffer (250 ml, 0.05 mM, prechilled to approximately 5°C) by blending in a Waring Blender (prechilled in a freezing cabinet at -20°C for 30 min). The nitrite and amine were added separately and blended for 1 min at high speed after the addition of each component. The mixture was frozen overnight (12 hr at -20°C) and then freeze dried for 24 hr in a Virtis RePP, Model No. 42 sublimator at a pressure of 5 $\mu$  and a platen temperature of 24°C. Residual moisture content was approximately 2%. Variations in the reaction mixtures, e.g., pH of the buffer system and amine/nitrite ratio depended on the parameter investigated and are described later.

#### Formation and extraction of N-nitrosamine from the freeze-dried system

The dried system was macerated to a fine powder and doubly wrapped in aluminum foil before heating in an oven at the required temperature for specified times. N-nitrosamine was extracted from the cellulose matrix by blending with 250 ml distilled water and 10g K<sub>2</sub>CO<sub>3</sub> for 1 min. The aqueous solution was extracted three times with ether (one 200 ml aliquot

followed by two 150 ml aliquots), shaking vigorously for 2 min for each extraction. The combined ether extracts were washed successively with 0.5M HCl and distilled water and dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> before concentrating to approximately 10 ml in a Kuderna-Danish evaporator. A final volume of 1 ml was obtained by evaporating the ether in a slow stream of nitrogen.

#### Gas chromatographic analysis

A Beckman GC-5 gas-liquid chromatograph equipped with a hydrogen flame detector and a stainless steel column (6 ft  $\times$  1/8 in. o.d.) packed with Chromosorb 101 (Johns-Manville Products Corp.), mesh size 80-100, was used for analysis. The chromatograph was operated isothermally at 215°C with gas flows of 25.6, 18.0 and 100 ml/min for nitrogen (carrier gas), hydrogen and compressed air respectively. Two  $\mu$ l aliquots of the ether concentrate were injected. Sensitivity of the apparatus was adjusted, varying with the initial concentrations of the reactants.

The amounts of N-nitrosamine formed in the system were determined using a previously prepared calibration curve.

### RESULTS

BEFORE INVESTIGATING the parameters controlling the reaction between secondary amine and sodium nitrite in a dry system, it was necessary to confirm that the compound formed was indeed a N-nitrosamine. This was achieved by thin layer chromatography (Griess reagent), gas chromatography using retention times on two columns (Chromosorb 101 and Carbowax 20M on Chromosorb W) and infrared analysis of the N-nitrosamine eluted from the TLC plates with spectrophotometric grade carbon tetrachloride. Further proof of N-nitrosamine formation in the dry model system was obtained by using a mixture of diethylamine, dipropylamine and dibutylamine instead of dibutylamine alone. Heating the freeze-dried model system resulted in the formation of the respective N-nitrosamines, DEN, DPN and DBN (confirmed by TLC and GLC analyses).

#### Parameters of the secondary amine/sodium nitrite reaction in the dry system

Among the various parameters investigated were the effects of time, temperature, pH and nitrite/amine ratio. The percentage recovery of DBN, added to 250 ml of a 1% CMC solution was also determined in triplicate (Table 1). Consequently all results presented were cor-



rected for losses due to incomplete extraction and concentration.

**Time and temperature effects**

A number of samples composed of 2.5g CMC, 0.25 mM NaNO<sub>2</sub> and 0.25 mM dibutylamine in borate buffer (pH 6.0) were prepared and dried as cited above. The samples were powdered, mixed in a rotating jar and then divided into the number of samples initially started with for testing in duplicate at each test condition. Three temperatures, 75, 100 and 125°C were used in the investigation and results are shown in Figure 1. Maximum formation of N-nitrosamine at 100 and 125°C occurred after 12 and 9 min respectively. However, maximum N-nitrosamine formation was not achieved after 2 hr at 75°C. This was determined by heating two similar samples for 30 min at 120°C when values of

3.32 and 3.38 mg DBN were obtained. There were small differences in the maximum amounts of N-nitrosamine formed at the various temperatures since the respective amounts of N-nitrosamine pres-

ent in the heated system is dependent on the retention of the secondary amine within the dried matrix during the freeze-drying process.

Temperature studies on samples heated

**Table 1—Percent recovery of dibutyl nitrosamine added to CMC solution**

Dibutyl nitrosamine (mg)	Percent recovery
2.5	89.5
2.5	88.8
2.5	87.0
5.0	86.4
5.0	86.4
5.0	87.5
5.0	86.5

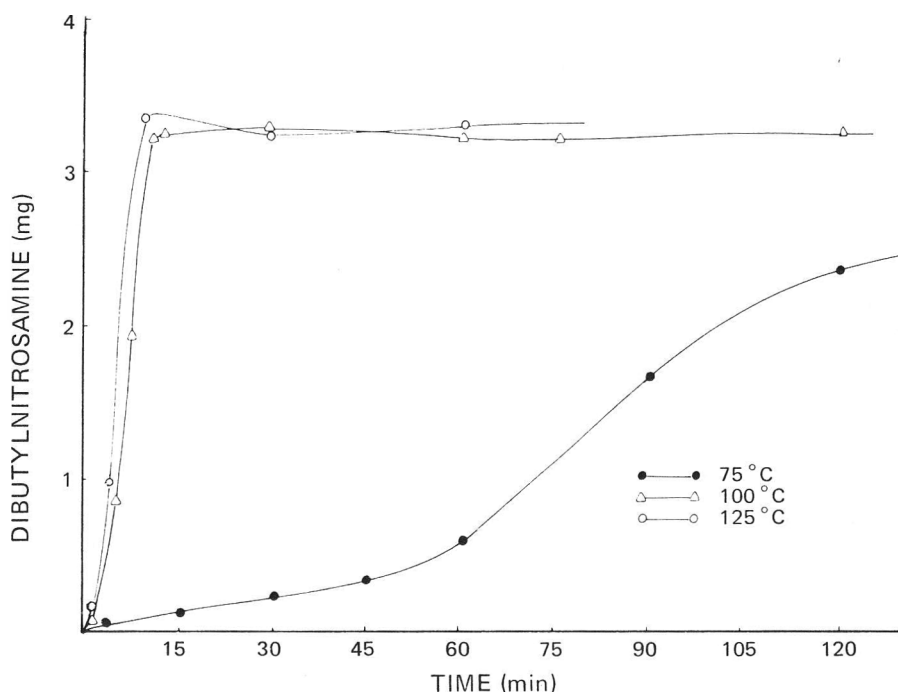


Fig. 1—Effect of time on the formation of dibutyl nitrosamine in the dry model system containing 0.25 mM dibutylamine and 0.25 mM sodium nitrite.

**Table 2—Formation of DMN during 24 hr at 22°C from 1 mM dimethylamine HCl and 1 mM NaNO<sub>2</sub> in 100 ml buffer at various pH values**

pH	Biphthalate/Phosphate buffer (mg DMN)	Borate buffer (mg DMN)
2.0	0.030	0.019
3.0	0.332	0.295
3.5	0.410	0.430
4.0	0.225	0.258
4.5	0.162	0.159
5.0	0.087	0.097
6.0	0.041	0.048
7.0	0.035	0.034
8.0	0.023	0.022

**Table 3—Effect of pH on DMN formation in aqueous systems containing (2.5g) 1 mM dimethylamine HCl and 1 mM sodium nitrite**

pH	Biphthalate/Phosphate buffer (mg DMN)	Borate buffer (mg DMN)
3.5	2.03	1.05
7.0	0.31	2.42

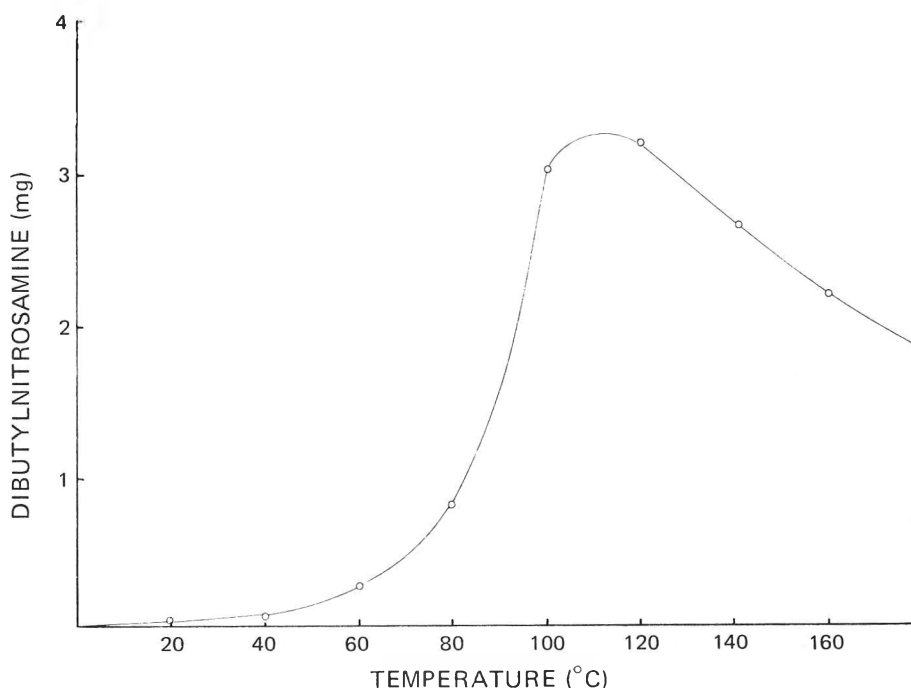


Fig. 2—Effect of temperature on the formation of dibutyl nitrosamine in the model system containing 0.25 mM sodium nitrite and 0.25 mM dibutylamine, time of heating 30 min.

for 30 min revealed maximum N-nitrosamine formation at 100–120°C with a rapid increase in its formation between 80 and 100°C. Some reduction in amounts formed was encountered at 140°C and higher temperatures (Fig. 2).

#### Nitrite/amine

The effect of nitrite on DBN forma-

tion in the low moisture CMC system (borate buffer, pH 6.0) was investigated using a fixed amine concentration (0.5 mM) in all the samples. These samples were heated at 100°C for 30 min. These studies revealed that maximum N-nitrosamine formation was not achieved until the nitrite to amine ratio was 2:1 or higher (Fig. 3). The higher ratios were not

effective in producing greater quantities of N-nitrosamine. The amount of N-nitrosamine is again dependent on the relative retention of the amine during freeze drying. Kjeldahl analysis and direct ether extraction followed by GLC analysis (Chromosorb 103) showed that 72–75% dibutylamine was retained during freeze drying.

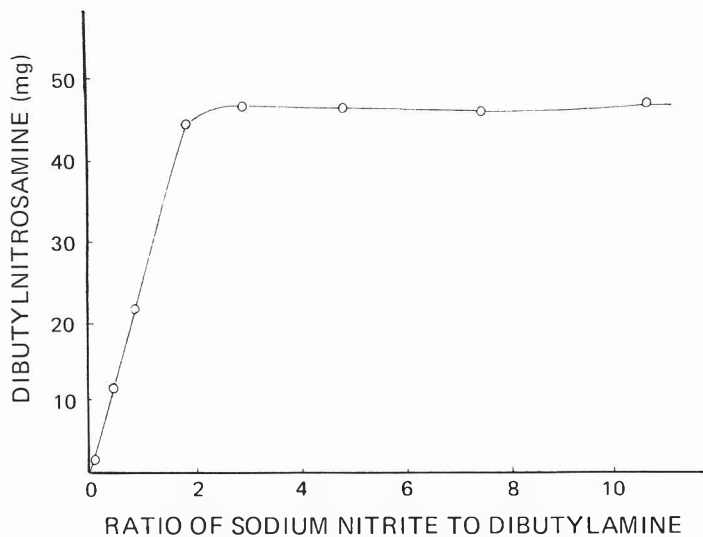


Fig. 3—Effect of sodium nitrite:dibutylamine ratio on the formation of dibutyl nitrosamine in a model system containing 0.5 mM dibutylamine.

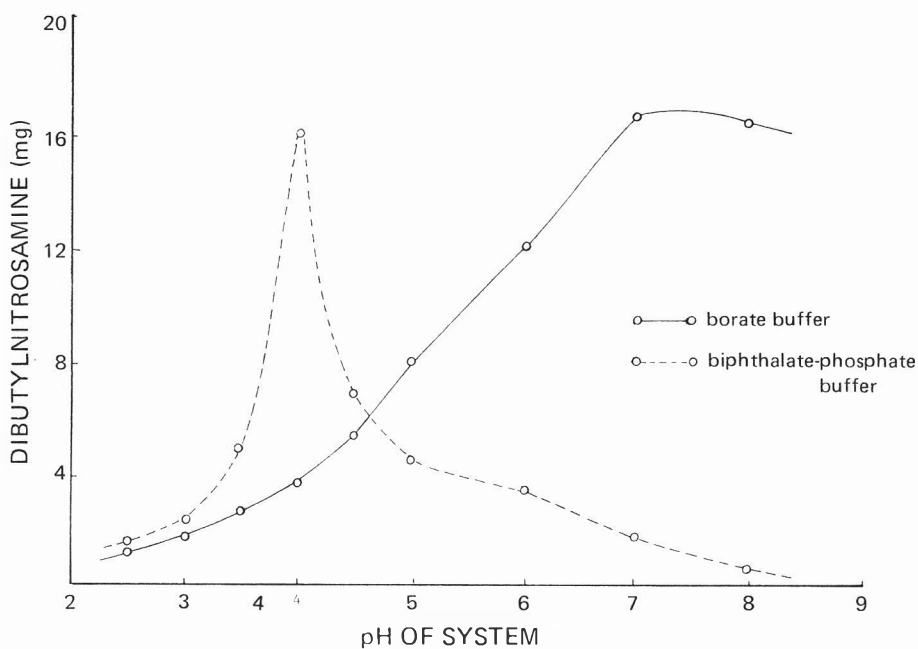


Fig. 4—Effect of pH (using two buffer systems) on the formation of dibutyl nitrosamine in the low moisture CMC system, reactant concentrations, 0.25 mM dibutylamine and 0.5 mM  $\text{NaNO}_2$ , temp 100°C.

#### pH effects

Using optimum reactant ratios (nitrite/amine 2:1), and heating for 30 min at 100°C resulted in the formation of increasing amounts of N-nitrosamine in the dried CMC system as the pH of the borate buffer system (before freeze drying) was varied from pH 2.5 to 8.0 (Fig. 4). A comparison shows 1.1 mg DBN formed at pH 2.5, 5.5 mg at pH 4.5 and 16.6 mg at pH 7.0.

The effect of pH on DBN formation in the dried system was also investigated using a phosphate/biphthalate system. Figure 4 shows maximum and minimum N-nitrosamine formation at pH 4 (15.8 mg) and pH 8 (0.5 mg) respectively. N-nitrosamine formation in the aqueous system of Ender and Ceh (1971) was also investigated using phosphate/biphthalate and borate as the buffer systems. Maximum formation of N-nitrosamine occurred at pH 3.5 for both buffer systems, then decreased with increasing pH (Table 2).

The influence of CMC on the formation of DMN in an aqueous system of 250 ml buffer (at pH 3.5 and 7.0), 1 mM  $\text{NaNO}_2$ , 1 mM dimethylamine HCl and 2.5g CMC was also investigated. The samples were shaken gently for 42 hr at 22°C, extracted with ether and concentrated to 1 ml, before quantitation using GLC analysis (Table 3).

#### DISCUSSION

THIS STUDY was initiated to establish if N-nitrosamines could be formed in dry systems similar to those encountered in dry foods or on the dry surface of cured meat products. The majority of the previous studies of the secondary amine/sodium nitrite reaction in model systems has been confined to aqueous solution (Ender et al., 1967; Ender and Ceh, 1971). However, the latter group has shown that N-nitrosamine formation can take place when certain amino acids and sodium nitrite are reacted together at elevated temperature (130, 160 and 170°C) in a starch matrix. In our CMC system, high concentrations of sodium nitrite were used in order to study the conditions under which maximum production of DBN was obtained.

Dibutylamine was chosen for the CMC reaction system because of its relatively high boiling point (159°C) as compared to dimethylamine (7.5°C), diethylamine

(55°C) and dipropylamine (110°C). Consequently, it would be expected that a greater percentage of dibutylamine would be retained within the cellulose matrix during freeze drying (Saravacos and Mayer, 1966). There would also be less amine expelled from the system when heated at elevated temperatures prior to its reaction with nitrite. One slight disadvantage in using dibutylamine as the secondary amine source instead of the lower homologues is that the yield of N-nitrosamine formed from secondary amine and sodium nitrite is much greater with weakly basic than with stronger basic secondary amines (Sander et al., 1968). Dibutylamine, having a  $pK_b$  value of 2.72 (Sykes, 1970), would be expected to be slightly less readily nitrosated than dimethylamine and diethylamine ( $pK_b$  values 3.23 and 3.07, respectively).

The time-temperature relationship, shown in Figures 1 and 2, indicates that both parameters are important in N-nitrosamine formation. This may have relevance to scattered observations reported in the literature that N-nitrosamines have been found in a food after cooking but not prior to cooking, e.g., cured bacon (Crosby et al., 1972). There appears to be a critical temperature at which N-nitrosamine formation is accelerated (80–100°C). A similar temperature effect has been reported by Ender et al. (1967) who found that DMN formation from dimethylamine hydrochloride and sodium nitrite in aqueous solution was approximately five times greater at 100°C than at 80°C.

It can also be seen from Figure 2 that maximum N-nitrosamine formation occurred at 100–125°C after which production decreased rapidly. This could be the result of either (1) decomposition of the N-nitrosamine or (2) expulsion of the amine from the system before it can react with the available nitrite. The latter explanation appears to be more acceptable as Fan and Tannenbaum (1972) have shown that dialkylnitrosamines are generally quite stable when heated at 110°C over a wide pH range. They also found that there was no reaction between N-nitrosamines and some chemical compounds common in food (glucose, ascorbic acid, cysteine, protein, etc.) after 3 hr at 110°C.

Since the temperature of 140°C is approaching the boiling point of dibutylamine, loss of amine from the freeze-dried matrix can be expected. This can be accelerated by partial degradation of the cellulose polymer as thermal decomposition of carbohydrates can be initiated at temperatures as low as 100°C (Murphy, 1962). It was also observed in this study that the amount of browning in the system increased with increasing temperature. This would indicate that carbonyl

compounds including glucose units are produced at the higher temperatures and these would participate in the nonenzymatic browning reaction with amine (Hodge, 1953).

The concentration level of nitrite and the nitrite to amine ratio are important and indicate that minimum or perhaps no N-nitrosamine formation may be expected when nitrite levels are low. Preliminary studies revealed that no N-nitrosamine could be detected (by GLC and TLC) in our system when permitted levels of sodium nitrite (200 ppm or less) and dibutylamine (ratio 2:1) were used. Maximum formation occurred at and above the nitrite to amine ratio of 2:1. However, the loss of amine during the freeze-drying process and browning reaction again must be taken into consideration and it is probable that a higher nitrite to amine ratio is necessary for maximum N-nitrosamine formation. Ender and Ceh (1971) reported that increasing the nitrite to amine ratio from 1:1 to 5:1 in the aqueous model system resulted in an approximately 16 fold increase in the relative amounts of DEN and DBN produced.

The effect of pH on the secondary amine/sodium nitrite reaction has been extensively studied using aqueous model systems. The reaction has been shown to be pH dependent and is favored by acid conditions, maximum N-nitrosamine formation occurring in the pH range, 3.4–4.0 (Ender and Ceh, 1971; Mirvish, 1970). These trends were confirmed in this study when the two buffer systems were used without the addition of CMC. A similar pH effect was also observed in the freeze-dried CMC system with the biphthalate/phosphate buffer, maximum formation occurring at pH 4.0. When CMC was added to an aqueous solution of the buffer containing the two reactants, the amount of N-nitrosamine formed at the two pH values, 3.5 and 7.0 varied with the buffer used (Table 3). The biphthalate/phosphate buffer displayed the expected pH trend, N-nitrosamine formation favoring the lower pH. However, with borate buffer, as in the freeze-dried system, a greater amount of N-nitrosamine was formed at pH 7.0.

Since the pH trends in both buffer solutions without added CMC are similar and vary with the addition of the cellulose matrix, it can be concluded that CMC plays an important role in modifying the reaction conditions. It was thought that the CMC molecule composed of glucose units perhaps formed chelates with boric acid and ultimately altered the pH of the system. However, this was disproved when solutions of CMC in borate buffer (1% w/v) at various pH values were prepared and no pH changes were recorded even after a week

at ambient temperature or 4 hr at 80°C. It can also be concluded that there is no reaction between boric acid and  $\text{NaNO}_2$  since the pH trend observed in the aqueous solution (without CMC) in this study agreed with the data reported in the literature. This reversal of the pH pattern (maximum N-nitrosamine formation at high pH) was observed only when CMC was present in the borate buffer system. It is possible that the molecules of the reactants orient themselves differently in the micelles of CMC in the borate buffer of varying pH. This could result in situations where the reactants are not readily accessible to each other. Independent studies have shown that the rate of formation of N-nitrosamine in the dried CMC system at pH 3.5 is approximately the same as that at pH 7.0. This implies that some of one or both reactants is being removed from the system. It is possible that a complex between boric acid (more abundant at low pH), cellulose and nitrite/amine can "tie-up" the reactants, so that the amount of N-nitrosamine formed is reduced. The boric acid and cellulose must both be involved in this sequestering effect since independent systems such as the boric acid/nitrite/amine system and biphthalate/nitrite/amine/CMC system yielded similar pH effects.

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## ANTIOXIDANT EFFECTIVENESS IN INTERMEDIATE MOISTURE CONTENT MODEL SYSTEMS

### INTRODUCTION

ALTHOUGH several authors have stated that lipid oxidation is not important at intermediate moisture content (Brockman, 1970, 1973; Loncin et al., 1968), studies of both model systems and intermediate moisture foods (IMF) show that rancidity occurs very rapidly and may be the limiting storage reaction (Labuza et al., 1969, 1971a, 1971b, 1972; Chou et al., 1973). In these cited works the importance of both water content and water activity ( $A_w$ ) was demonstrated in that as both increased well above the BET monolayer oxidation rates increase instead of decrease. This was shown to be due to mobilization of catalysts. In addition, systems prepared by adsorption of vapor oxidized slower than did desorption systems due mainly to the fact that at the lower water content the system had a higher viscosity and thus catalyst mobility was slower.

It was concluded from these studies that in order to insure long shelf life IMF would have to have antioxidants added along with control of the oxygen level by packaging techniques. Two types can be used; metal chelating agents which are water soluble and free radical fat soluble chain terminators like BHA and BHT. Several studies have been made to show the effect of these types of antioxidants as a function of  $A_w$ .

Tjho et al., (1969) and Labuza et al., (1971b) studied the effect of citric acid and EDTA as trace metal chelating agents as compared to BHA as a function of  $A_w$  in model systems. As expected, since both chelating agents are water soluble they were more effective as  $A_w$  increased to 0.61 in a cellulose based system. However, with protein present EDTA became partially tied up and was only as effective as BHA alone. When mixed in a semi-solid chicken or pork food at  $A_w$  0.75, BHA was much more effective than EDTA or citric acid as measured by both oxygen uptake and peroxide value (Labuza et al., 1971b, 1972). This, again, is probably due to the irreversible binding of the chelating agents to metals present in the protein making them ineffective.

Based on these studies it can be concluded that antioxidants can be used to control oxidation in the IMF range. For low protein foods such as cereals or

fruits, metal chelators should work best, whereas when protein content is high, free radical acceptors such as BHA should be more effective. The purpose of this study was to examine antioxidant effectiveness in carbohydrate systems as a function of sorption hysteresis, i.e., how the method of preparation of the system affects catalyst activity in a system where chelators operate best. In addition, a swelling support (amylopectin) with high initial metal content ( $\sim 1000$  ppm heavy metals) was tested against a nonswelling system (microcrystalline cellulose) to which metals were added. Previous work

(Chou et al., 1973) showed that the amylopectin system was a useful model system for studying lipid oxidation in relationship to foods which also swell as  $A_w$  increases.

### MATERIALS & METHODS

METHODS similar to that reported by Labuza (1971) and Labuza et al., (1972) were used. The system compositions used are shown in Table 1.

Glycerol and methyl linoleate were first mixed in a beaker using a glass stirring rod. To this the solid support (either microcrystalline cellulose (Avicel, FMC Corp.) or amylopectin was added and mixed thoroughly. For the direct mix system (DM) the amount of water necessary to achieve the desired water activity (as previously determined) was added. This gives the desorption system. For the humidified systems (DH) after mixing the support with the lipid and glycerol, the system was transferred into vacuum desiccators containing saturated salt solutions and held for 48 hr at 37°C. The isotherms were shown by Chou et al., (1973) indicating about a 5% moisture difference at similar  $A_w$ .

The antioxidants were added as follows: (1) For the fat soluble antioxidants (BHA, isopropyl citrate, tocopherol) the antioxidant was added directly into the oil. (2) For the water soluble antioxidants, these were dissolved in the water for the desorption system and in the glycerol for the humidified system. The systems tested are shown in Table 2. Cellulose II refers to the system with high metal content; cellulose I the system with only 100 ppm cobalt added.

Samples (in triplicate or duplicate) were prepared for oxidation studies by the method described above. The system was weighed directly into the Warburg manometer flasks before humidification to facilitate ease of handling. The direct mixed system was also held simultaneously in the desiccator so that the extent of oxidation is the same. Vacuum desiccators are used to prevent or to slow oxidation during the humidification procedure. After equilibration, the flasks were connected to the manometers and oxidation was measured. The method of Labuza (1971) was used to calculate oxygen uptake.

For measurement of effectiveness a control without antioxidant was run in each test. The induction time which is the time required to reach 3% oxidation [moles oxygen/mole linoleate] was determined from the oxygen uptake curve. When the oxidation was very slow (Runs 5 and 6) the time to 1% oxidation was used instead as the induction time. Antioxidant effectiveness was calculated as the ratio of the induction time divided by the induction time of the respective control.

Table 1—System composition model systems<sup>a</sup>

Microcrystalline cellulose system	(grams)
Methyl linoleate	10
Glycerol	40
Microcrystalline cellulose	50
Water (g H <sub>2</sub> O/100g solids):	
$A_w$ 0.68:	
DM	19
DH	17
$A_w$ 0.75:	
DM	32
DH	28
$A_w$ 0.84:	
DM	61
DH	49
$A_w$ 0.89:	
DM	74
DH	63
Amylopectin system	(grams)
Linoleate	10
Glycerol	40
(Starch) Amylopectin	50
Water (g H <sub>2</sub> O/100g solids):	
$A_w$ 0.68:	
DM	28
DH	15
$A_w$ 0.75:	
DM	32
DH	20
$A_w$ 0.84:	
DM	50
DH	29
$A_w$ 0.89:	
DM	71
DH	48

<sup>a</sup> DM = direct mix; DH = humidified mix.

RESULTS & DISCUSSION

THE KINETIC VALUES determined from the oxygen uptake data (average of 3 samples for each system) are shown in Table 3. Figure 1 is a partial summary of Runs 1 to 4 in which the induction times for the controls were normalized so that an easy visual comparison between systems could be made. In Table 4, the calculation of antioxidant effectiveness is presented.

As discussed above it has been shown that metal catalyst solubility and mobility were the most important reasons for the catalytic effect of water at high humidity. The top two sections of Figure 1 illustrate these results. In microcrystalline cellulose of low free metal content and amylopectin, the direct mixed desorption systems oxidize faster than the humidified systems. In addition, the oxidation rate increases as  $A_w$  increases. These increases can be attributed to the higher water content which swells the system and exposes new catalysts and a decrease in the liquid phase viscosity meaning greater mobility (Labuza and Chou, 1974). When free metals were added to the cellulose (Cellulose II in Fig. 1) to match the level in the amylopectin, however, the reversal occurs; i.e., the higher moisture system oxidizes slower with respect both to method of preparation and  $A_w$  due to dilution of the free catalyst in the available free water, as shown by Chou et al., (1973).

In the high metal cellulose model system the oxidation pattern followed for the EDTA-treated samples in Run 1 was similar to the control. The humidified system oxidized faster than the direct mix system and oxidation was faster at the lower  $A_w$ . Even though the EDTA is very effective, especially in the direct mix system, the pattern of oxidation does not change to that of a low metal system. Based on the induction time, EDTA reduces the rate by 10 to 20 times in the cellulose system (Table 4). The amylopectin systems show an effect similar to the cellulose system but opposite to that of the amylopectin system without EDTA, with respect to method of preparation. The effectiveness is also less as seen in Table 4, most likely because the metal present is more tightly bound. As seen, there is very little difference in oxidation rate with respect to  $A_w$  in the DM system. The change in rate with respect to water content and sample preparation method can be explained by the solubility of EDTA. As seen in the isotherms for EDTA (Fig. 2), solution begins to occur at about  $A_w$  0.94. Thus, when the direct mix system is made, the EDTA can chelate the metals in the aqueous solution. The metals are strongly chelated in both direct mix systems, giving an extremely slow oxidation rate. However, in the

Table 2—Run parameters

Run #	1	2	3	4	5	6
Solid supports:						
Cellulose II (1000 ppm metals)	X	X	X	X	X	
Amylopectin	X	X	X	X		
Cellulose I (100 ppm metals)					X	X
Water activity:						
0.68	X	X				
0.75	X	X	X		X	X
0.84	X	X	X	X	X	X
0.89					X	X
Control	X	X	X	X	X	X
Antioxidants tested:						
EDTA (0.08 moles/mole metal)	X					
Sodium citrate (0.5 moles/mole metal)		X	X			
Isopropyl citrate (0.5 moles/mole metal)			X			
Tocopherol (200 ppm fat basis)				X		
BHA (200 ppm fat basis)				X		
BHA (50 ppm)					X	
BHA (100 ppm)						X
BHA (100 ppm)						X
BHA/BHT (50/50 ppm)						X

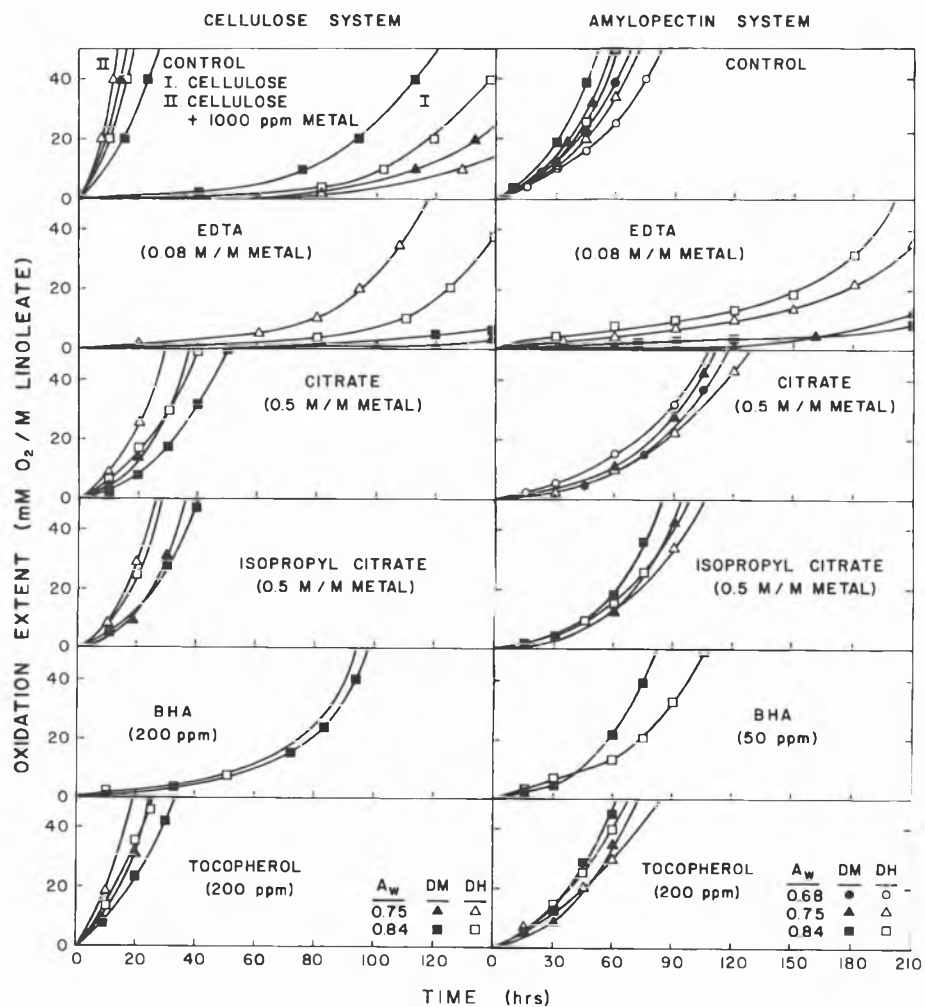


Fig. 1—Normalized oxidation extent at 37°C in model systems as a function of sorption hysteresis for various antioxidants. DM refers to desorption, DH to adsorption prepared systems.

humidified systems the higher the moisture content, the greater is the antioxidant effect. This means that the EDTA has greater solubility and mobility as the  $A_w$  increases. Also, the higher viscosity at the lower  $A_w$  should decrease the mobility of EDTA which is a fairly large molecule.

In Run 2, sodium citrate, another chelating agent, was tested. Its solubility point as shown in Figure 2 is at about  $A_w$

0.75. As seen in Table 4 and Figure 1, the citrate is a poorer antioxidant than EDTA, reducing the oxidation rate only by a factor of about 2 times. In the cellulose systems, the citrate is more effective at the higher  $A_w$  probably due to solubility of the citrate. However, the difference with respect to  $A_w$  is less since the solubility point is about  $A_w$  0.75, indicating that most of the citrate may have gone

into solution. This phenomenon is further illustrated in the amylopectin system. In the direct mixed system, the same pattern was followed as on the control. However, in the humidified systems  $A_w$  0.68 samples oxidized faster than those at  $A_w$  0.75. This can be explained by the solubility of citrate as was the case before with EDTA. Since citrate will solubilize at about  $A_w$  0.75 in humidified systems, it will be less effective at  $A_w$  0.68 due to its solubility. The citrate, as was the EDTA, is less effective in the swelling system (amylopectin) than in the cellulose as seen in Table 4. It should be noted that much more citrate than EDTA was used on a molar basis (about 6 times more). The maximum effectiveness occurred at the higher  $A_w$  studied.

Isopropyl citrate was studied in Run 3. It is partially water soluble and would tend to operate at the water-lipid interface and thus should be less affected by  $A_w$ . It was dissolved directly in the linoleate during mixing. The pattern follows the effects of  $A_w$  on the control in exactly the same pattern as for both solid supports. It must tie up some free metals initially and is not transported in the aqueous phase. Sodium citrate compared in the same run was about 50% more effective than the isopropyl ester on an overall basis as seen in Table 4.

In Run 4 and 5 both BHA and tocopherol were tested as antioxidants, both of which are free radical terminators having no effect on the trace metals. The oxidation rate followed the same pattern as in the controls for BHA, since BHA does not affect metal catalysts. It can be seen that BHA is very effective in the cellulose system at 200 ppm (Table 4). In the systems at a lower metal concentration and less BHA (50–100 ppm) the antioxidant is less effective as expected. In the amylopectin system, BHA at 50 ppm has about the same effectiveness as citrate. The results indicate that in cellulose BHA is more effective at the lowest moisture content, as was found by Labuza et al. (1971a). With amylopectin, the oxidation pattern is similar to that of the control but very little difference is observed for effectiveness as a function of  $A_w$ .

The d- $\alpha$ -tocopherol is fairly poor as an antioxidant as seen in Figure 1 and Table 4. In amylopectin its effectiveness is very small. Due to its expense and poor ability as compared to EDTA or BHA, it is not useful as an antioxidant; however, other forms of tocopherol should be tested.

With respect to antioxidants, the results of these tests indicate that BHA and EDTA, either alone or possibly in combination, would be very effective in controlling lipid oxidation at intermediate  $A_w$ . Further additional gain could be attained in vacuum packaging since the

Table 3—Lipid IMF model systems oxidation constants

Run #	Support	Antioxidants	$A_w$	Induction time (hr) <sup>a</sup>	
				Direct mix	Dry mix rehumidified
1	Cellulose <sup>b</sup>	EDTA	0.75	247	104
		EDTA	0.84	230	135
		None	0.75	11.0	9.7
		None	0.84	18.5	12.5
	Amylopectin	EDTA	0.75	250	198
		EDTA	0.84	261	177
		None	0.75	46.0	55.0
		None	0.84	38.0	43.5
		None	0.75	30.0	23.0
2	Cellulose	Sodium citrate	0.75	30.0	23.0
		Sodium citrate	0.84	40.0	31.0
		None	0.75	10.8	9.5
	Amylopectin	Sodium citrate	0.68	97.0	89.0
		Sodium citrate	0.75	92.0	102.0
		None	0.84	86.0	95.0
		None	0.68	53.0	67.0
		None	0.75	50.0	57.0
		None	0.84	86.0	95.0
3	Cellulose	Isopropyl citrate	0.75	29.0	20.0
		Isopropyl citrate	0.84	32.0	22.0
		Sodium citrate	0.84	42.0	34.0
		None	0.84	20.0	11.0
	Amylopectin	Isopropyl citrate	0.75	84.0	90.0
		Isopropyl citrate	0.84	76.0	85.0
		Sodium citrate	0.84	98.0	103
		None	0.84	43.0	54.0
		None	0.84	88.0	84.0
4	Cellulose	BHA	0.84	88.0	84.0
		Tocopherol	0.75	19.0	13.0
		Tocopherol	0.84	24.0	18.0
		None	0.75	12.0	9.0
	Amylopectin	None	0.84	19.0	15.0
		Tocopherol	0.75	56.0	59.0
		Tocopherol	0.84	50.0	47.0
		50 ppm BHA	0.75	47	68
		50 ppm BHA	0.84	46	46
5 <sup>c</sup>	Amylopectin	50 ppm BHA	0.89	40	45
		50 ppm BHA	0.75	293	340
		50 ppm BHA	0.84	226	311
	Cellulose	50 ppm BHA	0.89	168	299
		100 ppm BHA	0.75	372	498
		100 ppm BHA	0.84	349	435
6 <sup>c</sup>	Cellulose	100 ppm BHA	0.89	197	415
		100 ppm BHT	0.75	341	428
		100 ppm BHT	0.84	199	227
		100 ppm BHT	0.89	173	207
		50 ppm BHA plus			
		50 ppm BHT	0.84	344	473
		50 ppm BHT	0.84	344	473

<sup>a</sup> Time to reach 3% oxidation

<sup>b</sup> Microcrystalline cellulose

<sup>c</sup> Time to reach 1% oxidation measured

ratio of oxygen to lipid would be lowered to effectively slow the rate (Labuza, 1971). The results suggest that EDTA would be best in a low protein content food made by direct mixing (such as in extrusion processing of pet foods) if it is a

swelling system, which most are, whereas BHA is about equal in both systems. Thus, the combination should be very effective. The test also indicates that in the IMF region,  $A_w$  has only a small effect on antioxidant effectiveness, but rather moisture content is more important.

In Run 6 a study was made of the typical antioxidants added to intermediate moisture dog foods, namely butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) using the low metal cellulose system. As seen in Tables 3 and 4, the induction time is about 2 to 5 times longer than without antioxidant.

The same pattern of the oxidation rate being faster at higher  $A_w$  and the direct mix being faster than the humidified systems occurs in the presence of these types of antioxidants at 100 ppm added metals. This is because these antioxidants do not affect the metal catalyst concentration or catalyst mobility as do the chelating agents, but rather terminate free radical production. Thus, the changeover in kinetics does not occur.

In comparing the antioxidants in Table 4, BHA seems to be more effective especially in the humidified system. In combination, a 50/50 mixture of these two was as effective as 100% BHA. Since BHT is less expensive, the use of a mixture would be recommended.

In summary, the method of preparation resulting in sorption hysteresis has a pronounced effect on the effectiveness of metal chelating agents for inhibiting lipid oxidation. The systems prepared by desorption which have a higher moisture content oxidize slowest, probably because all the chelator is still in solution. Antioxidant effectiveness increases as  $A_w$  increases for the adsorption systems since it is expected that more chelator is dissolved as moisture increases. EDTA is more effective than BHA or BHT since the latter do not react with metals but rather are soluble in the lipid phase and act on free radicals only.

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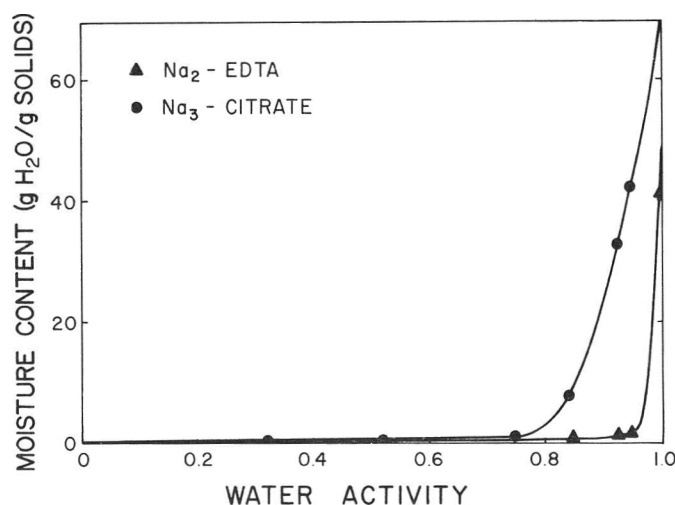


Fig. 2—Water sorption isotherms for sodium ethylene diamine tetraacetate and trisodium citrate at 37°C.

Table 4—Antioxidant effectiveness

System	Antioxidant	Effectiveness ratio <sup>a</sup>		
		$A_w$	DM <sup>b</sup>	DH <sup>b</sup>
Cellulose II (1000 ppm metals)	EDTA	0.75	22.5	10.7
		0.84	12.4	10.8
	Sodium citrate	0.75	2.8	2.4
		0.84	2.2	2.2
	Isopropyl citrate	0.75	2.6	2.1
		0.84	1.6	2.0
	$\alpha$ -tocopherol	0.75	1.6	1.4
		0.84	1.3	1.2
BHA (200 ppm)	0.84	4.6	5.6	
Cellulose I (100 ppm cobalt)	BHA (50 ppm)	0.75	3.2	3.1
		0.84	3.1	2.8
	BHA (100 ppm)	0.89	2.2	2.7
		0.75	4.0	4.6
	BHT (100 ppm)	0.84	4.8	4.0
		0.89	2.6	3.8
	BHA/BHT (50 ppm/50 ppm)	0.75	3.7	3.9
		0.84	2.8	2.1
Amylopectin	EDTA	0.75	5.3	3.6
		0.84	6.9	4.1
	Sodium citrate	0.68	1.8	1.3
		0.75	1.8	1.8
	Isopropyl citrate	0.84	2.3	2.0
		0.75	1.7	1.6
	$\alpha$ -tocopherol	0.84	1.8	1.6
		0.75	1.2	1.1
BHA (50 ppm)	0.75	0.84	1.3	1.1
		2.1	2.1	
		0.84	3.3	1.9
		0.89	2.5	2.0

<sup>a</sup> Ratio of induction time divided by induction time of respective control

<sup>b</sup> DM = direct mix; DH = dry mix rehumidified.



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## ON THE NATURE OF THE CHEMICAL AND PHYSICAL BONDS WHICH CONTRIBUTE TO SOME STRUCTURAL PROPERTIES OF PROTEIN FOODS: A HYPOTHESIS

### INTRODUCTION

THE PHYSICAL and chemical changes involved in alterations of meat quality, particularly tenderness, have been studied extensively during the past 70 years (Bate-Smith, 1948; Hamm, 1960; Dyer and Dingle, 1961; Fennema and Powrie, 1964; Szczesniak and Torgeson, 1965). Although it is believed that collagen in meat from beef can contribute a certain amount to a "background toughness" (Bailey, 1972), in fish meat collagen does not seem to participate greatly in the toughening process since fish muscle contains less connective tissue, only 3–4% vs. 2–27% of the total protein for mammals (Hamoir, 1961), and the collagenous material in the white muscle dissolves upon cooking and remains as a soft white mass after cooling.

Decreases in the amounts of proteins extractable with 7% lithium or potassium chloride solutions from beef and rabbit muscle which had been stored at temperatures from 0–35°C and fish muscle kept at a storage temperature of –3°C were first reported by Smith (1933); Bate-Smith (1937); Reay (1933); Reay and Kuchel (1936). However, undesirable changes in quality of cod, particularly transitions from tenderness to toughness were first correlated with the decreasing extractability of the actomyosin fraction by Dyer (1951). Luijpen (1957) found the solubility tests did not distinguish as well as organoleptic tests between cod samples frozen at –20°C and –30°C, but the correlation was very good for samples stored at –10°C. In beef the solubility of the fibrillar proteins were related to shearpress and taste panel evaluations (Hegarty et al., 1963). Weinberg and Rose (1960) found that the post-rigor increase in extractability of the contractile proteins of chicken meat paralleled an increase in tenderness while Khan et al. (1963) showed that the solubility of chicken muscle proteins decreased more rapidly during storage at –10°C than at –18°C. Using extraction methods somewhat different from those of previous workers, Goll et al. (1964) did not find any relation between protein solubility and tenderness.

Another approach toward the mechanisms of textural alterations was followed by Locker (1960), who related sarcomere length of the myofibrils to tenderness. These findings were confirmed by Herring et al. (1965) who found that as the post-mortem muscles shortened there were corresponding decreases in sarcomere length, increases in fiber diameter and decreases in tenderness, and if the excised muscles were restrained from postmortem contraction they would remain more tender. In view of the sliding filament model (Huxley, 1963; Huxley and Brown, 1967) in which the two major structural proteins, actin and myosin, are arranged in a highly organized superlattice of thin and thick filaments, their postmortem interaction at diminishing ATP concentrations, the increasing cell disorganization with the breakdown of membrane integrity, the failure of the calcium pump in the absence of ATP and the unrestricted movement of ions including those of heavy metals are some of the factors which gradually lead to the random interaction between cell constituents. These mechanical considerations then also make it tempting to relate the extent to which the thick filaments, equipped with "cross bridges," overlap or interdigitate with the thin filaments during the shortening phase of the contraction process and during rigor mortis (Huxley, 1956) to the chemical aspects of protein-protein interactions and to meat texture. Despite the fact that the extractability tests were not found entirely suitable for practical applications, the study of particularly the two major structural proteins actin and myosin, their self-associating behavior as well as the product of their interaction, the actomyosin complex, would appear necessary for an understanding of meat texture and the possibility of its chemical manipulation.

Ultracentrifugal studies of the mechanisms involved in the "spontaneous aggregation" of myosin in solution were first carried out by Holtzer (1956). This aggregation phenomenon had been a recognized problem accompanying myosin purifications from their earliest beginnings (Portzehl et al., 1950). Further physical studies on the aggregation of myosins, including that from fish, were carried out by Holtzer and Lowey

(1959), Connell (1959, 1960) and Johnson and Rowe (1961) who generally studied the rate of the decreasing monomeric myosin concentration with increasing time. Results from these experiments showed that myosin aggregation at 0°C increased with increasing protein concentration, was at a minimum around pH 7–8, and increased with increasing ionic strength. The sulfhydryl (SH) groups of myosin were also investigated as possible contributors to the aggregation reactions; however, it was concluded that they were not involved in the bonding which holds the myosin aggregates together. The reasons for this conclusion were given as follows: (1) The numbers of SH groups measured with N-ethylmaleimide did not change during 7 days of storage at 0°C and myosin reacted with N-ethylmaleimide (blocked SH groups) aggregated as rapidly as untreated myosin; (2) Monothioglycol at 0.01M did not inhibit aggregation (Connell, 1959) and at a concentration of 0.1M monothioglycol rather accelerated aggregation (Connell, 1960).

Investigations to elucidate the nature of the interactions between proteins which may contribute to the structural properties of meats and other foods were therefore continued.

### EXPERIMENTAL

#### Solid egg white

Solid egg white from a "5 min egg" (4g) was chopped into small particles about 0.5 cm on edge and was then suspended in 30 ml of 6M guanidine HCl which had been adjusted with 1.0N KOH to pH 10.5. The particles only swelled in this solution and the opaque white changed to a somewhat more translucent appearance during 24 hr of storage at room temperature. After the addition of reducing agent, 3 × 0.2g portions of NaBH<sub>4</sub> and readjusting the pH with KOH, the protein began to dissolve to a clear solution within about 1 hr. Addition of an octanol, methyl-n-hexylcarbinol, was used to reduce foaming problems.

Using mercaptoethanol as the reducing agent instead of NaBH<sub>4</sub>, the solution was prepared so that it was 6M in guanidine HCl, 0.5M in mercaptoethanol, and was then adjusted to between pH 8.5 and 9.0. After dissolution, the protein could be precipitated again by dialysis or by dilution and acidification.

The 6M guanidine HCl solution was prepared by dissolving 17.28g of guanidine HCl ("ultra pure" reagent from Schwarz-Mann) in about 10 ml of water, adding 2.2 ml N KOH

<sup>1</sup> Present address: Canada Dept. of Agriculture Experiment Station, Summerland, B.C., Canada

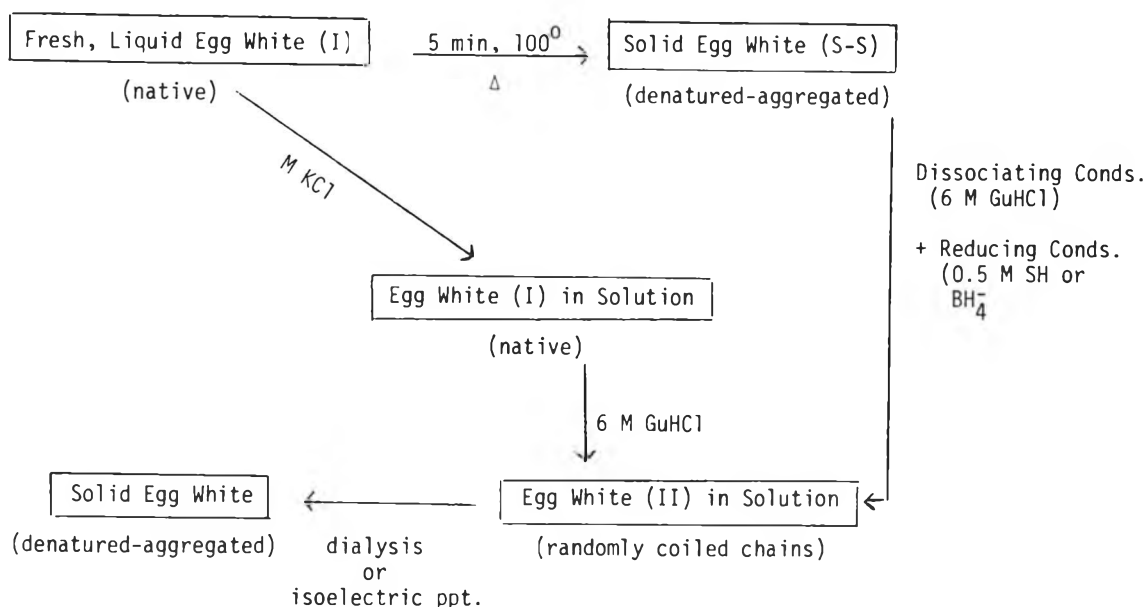


Fig. 1—Schematic diagram of the various physical states of egg white. Heat transforms liquid egg white, which consists of 54% ovalbumin, an SH protein, into a solid, firm or gelatinous form. This denatured, aggregated egg white ( $\beta$ -structure, fully extended peptide chain) cannot be solubilized in a nonhydrolytic way at room temperature by salt (KCl) solutions which are acidified (pH 1.0) or made basic (pH 12.0) nor by 6M guanidine hydrochloride (GuHCl) solutions (pH 10.0).

In order to bring the solidified or gel-like structure of the egg white (S-S) back into solution, conditions are required which will dissociate noncovalent hydrophobic, hydrogen and electrostatic bonds as well as

bring about reduction of covalent SS bonds which have formed during the heating process. These dissociating and reducing conditions are provided by a solution which is 6M in GuHCl, 0.5M in mercaptoethanol and at pH 9.0–9.5. When sodium borohydride is used as a reducing agent, the pH is maintained at 10.5 with M NaOH. In the dissociating-reducing solvent the egg white proteins are present as randomly coiled polypeptide chains and can be regenerated back to a solid, denatured form by dialysis or by dilution and acidification (isoelectric precipitation). Dialysis of "Egg White (II)" against a solution of KCl containing reducing agent will permit the proteins to regain the native form of "Egg White (I)."

and making final adjustments to pH 10.5 with 5 and 1N KOH before the volume was made up to 30 ml with water. If the solution was to be 6M in guanidine HCl as well as 0.5M in mercaptoethanol, 17.28g of guanidine HCl were dissolved in about 10 ml of water and 2.2 ml N KOH, 1.17g mercaptoethanol and two pellets (about 0.3g) of sodium hydroxide were added and dissolved before adjusting the volume to 30 ml with water. The pH of this latter solution was then generally around pH 9.0. Sodium bisulfite was not a suitable substitute for  $\text{NaBH}_4$  or mercaptoethanol due to its relatively low solubility in the already concentrated solution. However, sodium bisulfite has been used previously in combination with sodium dodecylsulfate at 100°C in the dispersion of 60–80% of chicken feather keratin (Lundgren and O'Connell, 1944).

#### Lingcod muscle

Lingcod muscle was cooked in a microwave oven or pressure cooker. About 5g of this cooked meat was then chopped into small pieces, extracted two times with diethyl ether and evacuated with a water pump to remove residual ether. The fibrous material was then suspended in 30 ml of 6M guanidine HCl adjusted to pH 10.5. After about 30 min of standing at room temperature, a gel had formed which could be broken gradually by the addition of mercaptoethanol adjusted to pH 9.0 but more effectively with  $\text{NaBH}_4$  at pH 10.5. The resulting solution, however, remained turbid

and had to be clarified by centrifugation at about 20,000  $\times$  G. After centrifugation a layer of material, presumably lipoprotein, was floating at the top of the tube. This material constituted about 4% of the weight of the original meat. The protein dissolved in the guanidine-reducing solution could be brought out of solution back into the solid state by dialysis or acidification to the isoelectric point of the proteins.

#### Kamaboko gel

Kamaboko gel (about 4g) appeared to dissolve to some extent in 30 ml 6M guanidine HCl solution at pH 10.5. However, after 24 hr the dissolution was still incomplete. To completely dissolve the material the 6M guanidine HCl solution had to be also 0.5M in mercaptoethanol at about pH 9.0. Lipoprotein material causing turbidity in the solution floated to the top within 24 hr or could be removed by centrifugation. On dialysis the protein precipitated again and remained as a white foam-mat type of material after freeze drying.

#### Beef

Beef (cooked as roast) was cut into 0.5 cm cubes. When 2.5g were added to 30 ml of the guanidine HCl-mercaptoethanol solution at pH 9.0, the meat particles became translucent and turned a blood-red color as the dissociating-reducing solution penetrated the meat, dissolved the proteins and at the same time reduced the ferric ion of metmyoglobin to the ferrous state in myoglobin. After 10 hr, about

36% of the initial material was still undissolved and consisted mostly of red muscle fibers embedded in collagenous material. When this material was finely ground up, more complete dissolution was achieved with only small amounts (1–2%) of presumably collagen and elastin, as judged from their blue-white fluorescence (Ex 360 nm) remaining. It was therefore concluded that the degree of dispersion must be related to the rate of solution particularly of the preparations containing hydrophobic membranous lipid materials and collagenous or cross-linked proteins.

Myosin was prepared, aggregated and redissolved as previously described (Buttkus, 1970; 1971).

## RESULTS & DISCUSSION

THE EXPERIMENTS of Connell (1959, 1960) tested for the involvement of the SH groups in the denaturation mechanism of myosin by adding mercaptans as protective agents after the extraction of the protein. However, the negative results did not exclude the function of the SH groups from a more complex series of mechanisms in which other forms of molecular interactions as well are participating in the aggregation phenomena. For instance, a molecular alteration brought about by the formation of disulfide (SS)

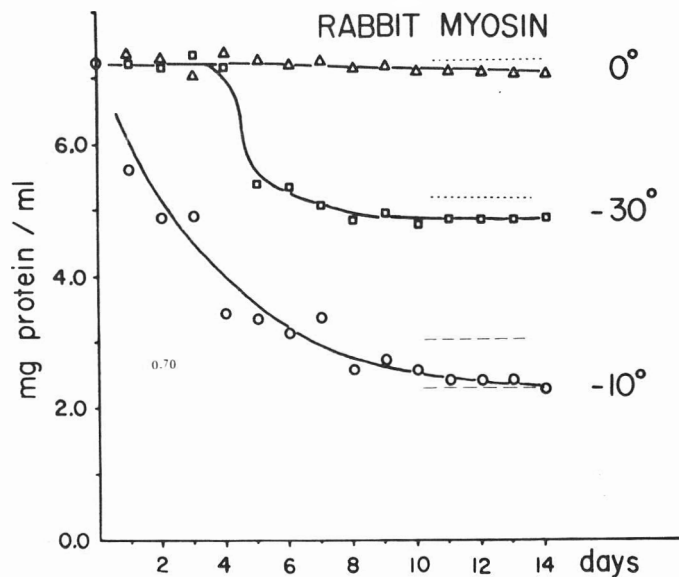


Fig. 2—Relative rates of decreasing protein concentration in myosin solutions stored at 0, -10 and -30°C for up to 14 days (solid lines). The myosin solutions were 0.72% in protein, 0.45M in KCl, buffered with 0.026M potassium phosphate at pH 6.9. After freezing, the protein solutions were thawed in 4–5°C water centrifuged at 12,000 X G and the remaining protein in the supernate was measured. The differences between the dashed and solid lines indicate the ranges of denaturation contributed by the catalysis of heavy metal contaminations (about  $2 \times 10^{-3}\%$ ) present in 'reagent grade' KCl, above the aggregation caused by the concentration effect of the protein during freezing. The values for the dashed lines were derived from Fig. 3 and superimposed on the rates of myosin aggregation at different temperatures and consequently different salt concentrations. For instance, in the liquid regions of the myosin solution frozen to -10°C, the KCl concentration can be expected to be about 3.2 molar (from a temperature-composition phase diagram of KCl). In the solution held at 0°C no concentration of the salt or protein will take place.

bonds followed by a rearrangement of hydrophobic and hydrogen bonded regions on an intra- and intermolecular basis during denaturation and aggregation could not be reversed by SS bond reducing agents alone, but would have to include conditions which would also break intermolecular hydrophobic and hydrogen bonds. From our studies on aggregated myosins, it was concluded that such complex interactions are indeed involved and must be very similar to those in heat denatured egg white (Fig. 1). However, selective conditions for the reversal of the "random" noncovalent bonding in the aggregated state or from the randomly coiled peptide chains to the organized arrangement in the native protein have not been worked out for egg white or myosin as it has in the case of lysozyme (Tanford et al., 1966).

In contrast to the denatured-aggregated myosin which requires hydrophobic, hydrogen as well as SS bond breaking reagents for its unfolding, in the depolymerization of the enzyme urease

only SS bond reducing reagents seem to be required as the polymeric species can be reversed to a monomeric form by the addition of low concentrations (0.01M) of reducing agent (Creeth and Nichol, 1960). In gelatin primarily hydrogen bonds between the polypeptide chains are responsible for its water including gel structure which can be ruptured already at 20°C upon the addition of 2M salt (KCl) solution pH 5–7 or by heating to higher temperatures in the absence of solvent. The structure of acid precipitated casein is believed to be held primarily by electrostatic bonding and can be dissociated again in 1M KCl solution at pH 12 to a milky solution. Extensive studies on gelatin interactions and the casein micelle formation are recorded in the literature (Harrington and Rao, 1970; Waugh, 1961). Yet another type of aggregation takes place in the case of tobacco mosaic virus protein, whereby monomers aggregate with a release of water molecules when the temperature is raised from 5°C to 25°C and the reaction is reversed on

lowering it (Lauffer et al., 1958).

While previous workers had concentrated their studies on the monomeric, native myosin remaining in solution after denaturation, in more recent work (Buttkus, 1970), the properties of the insolubilized, aggregated or polymeric myosin which can readily be centrifuged out of solution were studied. In a high ionic strength solution of 0.5M KCl, the insolubilization of myosin from rabbit or trout muscle proceeded at a faster rate when the solution was frozen than at 0°C (Fig. 2). To reverse the reactions of aggregation and bring the insoluble aggregated proteins back into solution, two general types of conditions had to be provided: namely, one to break or weaken the noncovalent associations, particularly the hydrophobic and hydrogen bonds, using guanidine HCl, and the second to break the covalent SS bonds by the use of reducing agents such as mercaptoethanol or sodium borohydride. While native myosin will unfold and dissociate into randomly coiled peptide chains in 8M urea solution, the aggregated polymeric myosin, which is insoluble in salt solutions, redissolved in 6M guanidine hydrochloride (pH 8.0–10.5) only after the addition of reducing agents. These results indicated the involvement of SS as well as noncovalent hydrogen, hydrophobic and possibly ionic bonds in the formation of the polymeric myosin. From the changes in the SH content of a myosin solution during storage, the initiating mechanism in the myosin polymerization was postulated to involve first an intramolecular SS bond formation by oxidation of up to 5–6 of the 42 SH groups in the native, monomeric molecules ( $5 \times 10^5$  g per mole) and proceeded then by intermolecular SH-SS exchange reactions involving the remaining free sulfhydryl groups on neighboring molecules. It can be visualized that during the denaturation processes the hydrophobic and hydrogen bonds buried in the interior of the protein molecule become exposed and broken from their native arrangement following conformational changes in coiled or helical sections of the peptide chains (Morawetz, 1972), and reform in a manner different from those in the native structures, possibly also intermolecularly.

The results of previous workers that N-ethylmaleimide or low concentrations of reducing agents had no protective effects on myosin aggregation was confirmed in our studies. However, these tests cannot be considered conclusive evidence for the critical involvement of SH groups in the denaturation mechanism since it is known that without the addition of denaturing agents, such as urea or guanidine, only a portion of the SH groups of myosin are available for reaction with these reagents (Buttkus, 1971). Myosin molecules, on the other

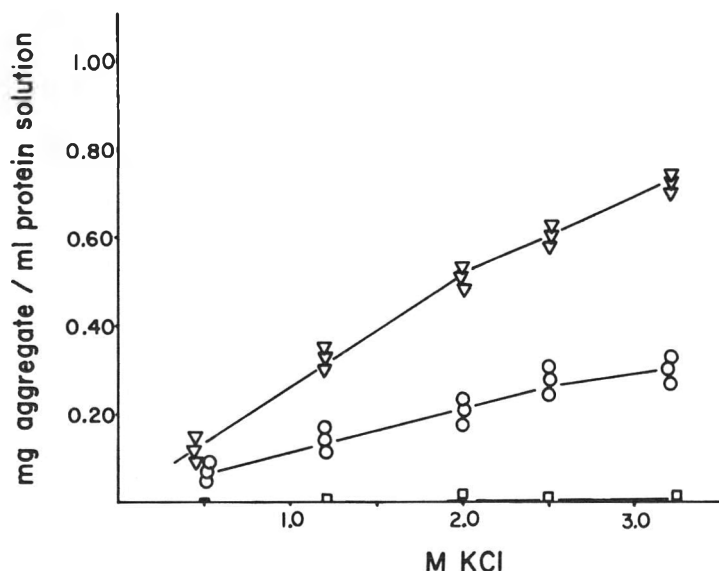


Fig. 3—Effect of different salt concentrations, 0.5–3.3M 'reagent grade' KCl, on the aggregation of rabbit myosin during storage periods of 3 days (open circles) and 12 days (triangles) at 0°C. The initial protein concentration in the different samples was 0.75% (7.5 mg protein per ml). Myosin prepared with salt solution which had previously been passed over a chelating resin (Chelex 100) showed very little to no aggregation in 0.5–3.2M KCl after 14 days of storage at 0°C (squares).

hand, may exert an action like denaturing agents upon their neighbors and gain thereby access into the interior of each other. Further, the fact that relatively low concentrations of mercaptoethanol produced an acceleration of myosin aggregation strengthened our belief that after a few days of storage or sometimes already during or after preparation some myosin molecules contained SS bonds. These considerations were in agreement with the experiments of Huggins et al. (1951), who, using plasma albumin or  $\gamma$ -globulin, both proteins containing SS as well as SH groups, showed that the addition of small amounts of thiols enhanced the rate of intermolecular SH-SS exchange, leading to the formation of polymers connected by SS bonds.

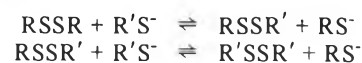
Studies on the rate of aggregation of myosin in frozen solution showed that it proceeded fastest at  $-10^{\circ}\text{C}$  in the vicinity of the eutectic point of the potassium chloride solution which was used to solubilize the native protein. On lowering the temperature from the freezing point down to the eutectic point, the freezing of water as ice results in the concentration of solutes such as buffer, salts as well as proteins, and reaches an optimum effect at the eutectic point. Since the rate of bimolecular or multimolecular reactions are concentration dependent, the finding that the maximum rate of aggregation of myosin was around  $-10^{\circ}\text{C}$ , the eutectic point of the solvent solution, was

in agreement with the theories of Kiovisky and Pincock (1966) who had shown that for systems for which the kinetics were already known at ambient temperatures the accelerated rates in frozen solutions could be predicted on the basis of the concentration of solute in the liquid regions of the apparently solidly frozen system. The proposed mechanism for the accelerated rate of aggregation of the salt-soluble contractile muscle proteins in the liquid regions of the frozen solution is further supported by the findings of Connell (1960) who showed that the rate of myosin aggregation at  $0^{\circ}\text{C}$  increased with increasing protein concentrations.

The results of our investigation on the effect of salt (KCl) on myosin aggregation agreed essentially with those of earlier publications (Holtzer and Lowey, 1959; Connell, 1960) and showed that increasing concentrations of reagent grade KCl increased the rate of myosin aggregation (Fig. 3). However, myosin prepared with the greatest possible care to exclude trace metal contaminations from reagents (i.e., iron content of crystalline KCl was  $1 \times 10^{-6}\%$ ), glassware and water with an electrical resistance of 18 megohms-cm did not show this effect of increased rate of aggregation with increasing salt concentration. It was further shown (Buttkus, 1971) that some of the trace metals present in reagent grade chemicals formed mercaptides with the SH groups of myosin and, while some of the metals

could be removed with chelating agents, the total number of SH groups was generally 5–6 moles SH per  $5 \times 10^5$  g of protein lower than in myosins prepared with reagents which had previously been purified by passage over chelating resins. The decrease in SH groups detectable with the Ellman reagent in the presence of chelating agent was therefore attributable to a metal catalyzed formation of SS bonds. These SS bonds are then able to undergo exchange reactions with the remaining SH groups in neighbouring molecules to form intermolecular covalent bonds, a major step in the aggregation polymerization process.

An important consideration in this SH-SS exchange mechanism during the denaturation of myosin is that once 2–6 SH groups of the original 42 per molecule have been oxidized, the polymerization process can proceed without any further decrease in measurable SH groups because for every SH group which then becomes incorporated into an SS bond another SH or mercaptide group ( $\text{S}^-$ ) is generated by an established mechanism (Cecil and McPhee, 1959).



A representative example of the rate of myosin denaturation or insolubilization at different temperatures is shown in Figure 2. The protein was dissolved in KCl solution prepared with 'reagent grade' KCl (containing  $1 \times 10^{-4}\%$  iron) and glass-distilled water. The contribution of metal ions to aggregation in the eutectic point region ( $-10^{\circ}\text{C}$ ) was obtained from Figure 3 by taking the difference between the amount of protein aggregate formed after 12 and 14 days of storage at  $0^{\circ}\text{C}$  in solutions of 3.2M 'reagent grade' KCl and 3.2M 'Chelex 100' treated KCl which contained  $1 \times 10^{-6}\%$  iron and was dissolved in 'Millipore' Super-Q water of lowest conductivity (18 megohms-cm). As can be seen in Figure 2, aggregation of the protein myosin due to metal contamination from the reagents plus the denaturation due to the concentration effect of the protein in frozen solution is represented by the solid line while the denaturation in solutions with a 100 $\times$  reduced metal contamination, broken lines, represents the level of myosin aggregation caused primarily by the concentration effect on the protein during freezing. The difference is about 8% of the total protein (7.2–8.0 mg/ml) originally present in solution and does not alter the relative order of the aggregation rates during freezing,  $-10^{\circ} > -20^{\circ} > -30^{\circ} > 0^{\circ}\text{C}$ .

The mechanisms and types of bonds outlined above in the denaturation and aggregation of purified myosins appeared to be of a more general nature and were shown to be also involved in the denatur-

ation and aggregation processes during the cooking of foods containing sulfhydryl proteins such as egg white, kama-boko or whole muscle. The cooked products which were firmer and had a greater shear strength than the raw materials (Buttkus, 1963) could be solubilized in 6M guanidine HCl only in the presence of reducing agents such as mercaptoethanol or borohydride, indicating the critical involvement of covalent disulfide as well as noncovalent hydrophobic, hydrogen and probably ionic bonds in the firming process of these foods during heating. In order to achieve the dissolution of cooked, firm egg white in about 1 hr at 20°C, the dissociating solution was made 0.5M in the reducing agent mercaptoethanol. The sodium borohydride reagent was used at a concentration of about 2% (0.4–0.5M). Sodium sulfite was not a preferred reducing agent due to its poor solubility characteristics in the already concentrated GuHCl solution. Figure 1 shows a schematic diagram of the various physical states in which egg white, composed of the various proteins [ovalbumin 54%, conalbumin 13%, ovomucoid 11%, lysozyme 3% and others (Feeney, 1964)] can exist and the necessity of reducing agents to cleave the SS bonds of the solidified, hard or denatured egg white as well as reagents to provide conditions for the dissociation of hydrophobic and hydrogen bonds to bring about dissolution. The heat denatured, solidified egg white would not dissolve in 2M KCl containing reducing agents, neither will it dissolve at room temperature in salt solutions which have been acidified to pH 1 or made basic to pH 12. The solubility properties of heat denatured egg white therefore appear similar to those of denatured myosin and a substantial fraction of the keratins has also been reported to yield to extraction at 40–50°C with saturated urea containing reducing agents. By x-ray analysis, regenerated keratin, once of interest to the textile industries, and heat denatured egg white were both found to have a  $\beta$ -keratin type, unfolded or denatured structure (Wormell and Happey, 1949). The formation of SS bonds during the heating of dilute solutions of ovalbumin had also been suggested by Halwer (1954) and our evidence that reducing conditions are required in the dissolution of the solid egg white further supports the earlier findings of Hamm and Hofmann (1965) that SS bonds are formed during the cooking of meat and that they possibly contribute to the firming or toughening in the cooking process. The unique elastic and cohesive properties of wheat proteins have also been attributed to their disulfide bonds (Nielsen et al., 1962). On the other hand, a gel containing 12% gelatin, a sulfur-free protein having a low content of amino acids with hydrophobic side chains, readily dissolved

at room temperature in 2M KCl or 8M urea, and no reducing agent was required.

Whole tissues containing membranous and other lipid material were much more difficult to dissolve than egg white, kama-boko, or denatured myosin, probably due to poor penetration of the aqueous solvent into the lipid material containing also insoluble residues such as collagen and elastin. Particularly in fishery products some insolubility of lipid-containing material after long or poor storage conditions may also be due to the interaction of the proteins with autoxidation products from polyunsaturated lipids such as aldehydes, ketones, peroxides and epoxides, resulting in products which are cross-linked and have solubility properties similar to the naturally cross-linked proteins collagen or elastin (Buttkus, 1970). Similar effects on the solubility of proteins are also produced by formaldehyde, a product from the reduction of trimethylamine oxide in some species of fish (Castell et al., 1973).

When, therefore, raw or cooked fish muscle slices were dissolved under the above dissociating and reducing conditions of 6M guanidine HCl and 0.5M mercaptoethanol or NaBH<sub>4</sub>, some distinct differences were noted in comparison with the behavior of cooked egg white and denatured myosin. The solutions obtained after reduction and dissociation of the SS and noncovalent bonds were turbid instead of clear as the ones obtained from egg white. The lipid material could, however, be separated from the remaining solution upon centrifugation when a lipid or lipid-protein layer floated to the top. The protein in solution could then be reclaimed as a precipitate on acidification or dialysis. Due to poor penetration of the solvent into the tissue, the speed of dissolution of cooked muscle, particularly that from beef, was very much dependent on its degree of dispersion, i.e., the finer emulsified the muscle, the faster it would dissolve. Cooked egg white would go into solution readily when it was chopped up to about 0.5 cm cubes and left in the dissociating-reducing solution for 1 hr at 20°C.

That 95% of undenatured protein can be extracted from fresh fish muscle with 0.85M NaCl after homogenization in a Waring Blendor has been reported by Dyer et al. (1950). Tomlinson and Geiger (1963) have shown that, if the extraction is carried out in metallic (stainless steel) vessels, quite large decreases in the extractable protein occurred. A reduction in the rate of myosin aggregation in the presence of polyphosphates (Buttkus, 1970) and the successful use of polyphosphates in the 'tenderization' of meat (Ellinger, 1972) suggest a general mechanism involving chelation of metal ions by the phosphates. The addition of polyphosphates to protein solutions can there-

fore prevent further formation of SS bonds but it cannot cleave those which have already formed. In terms of whole meat systems this would mean that the addition of polyphosphates can prevent further toughening involving the formation of SS bonds, but could not make extensively cross-linked or tough meat more tender.

Observations made on the SH groups of the purified proteins myosin and actomyosin during their preparation storage and denaturation (Morales and Hotta, 1960; Blum, 1960; Buttkus, 1971) appeared to be in general agreement with the behavior of the natural products containing sulfur proteins such as egg white and meat from fish and mammals. Metal catalyzed oxidation of the SH groups and a reversal by cleavage of some of the SS bonds also by metals (Cecil and McPhee, 1959) appears to be responsible for the erratic results which are sometimes obtained in the measurement of the SH content. This has been particularly demonstrated in actomyosin preparations where fluctuations within a relatively short time period cycled from 6.0 to 5.0 and back to 6.0 SH groups per 10<sup>5</sup>g of rabbit myosin B (Blum, 1960). For chicken actomyosin variations from 9.8 to 8.7 and back again to 9.4 SH per 10<sup>5</sup>g with a standard deviation as high as  $\pm 0.8$  have been reported by Hay et al. (1972). At an approximate molecular weight of  $5 \times 10^5$ g of protein for myosin, a decrease by about 1.0–1.2 SH groups per 10<sup>5</sup>g would indicate that about 5–6 SH groups have been oxidized to 2.5–3.0 SS bonds per molecule and the presence of SS bonds in the protein molecules would then enable them to react by intermolecular SH-SS exchange reactions with the remaining SH groups of other molecules to form polymeric material. In work on myosin, prepared with purified reagents (Chelex 100 treatment), a standard deviation of  $\pm 0.11$  moles SH per 10<sup>5</sup>g was generally attained with the Ellman procedure which made it possible to measure the SH content per myosin molecule with a variation of not more than  $\pm 0.55$  SH residues. These values are in agreement with work on actomyosin, prepared with purified reagents, where SH per 10<sup>5</sup>g of protein were measured with a standard deviation of  $\pm 0.1$  (Jacobson and Henderson, 1971).

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## ESTIMATION OF CHANGES IN THE AVAILABILITY OF EACH INDIVIDUAL ESSENTIAL AMINO ACID IN FOOD PROTEINS

### INTRODUCTION

WITH AN EVER INCREASING consumption of processed foods and especially of foods containing highly processed proteins, a great need exists for improved methods to assess the effects of processing on the nutritional availability of the amino acids supplied by such foods. A variety of biological methods, including bioassays, have been used to study the availability of amino acids of food proteins (see the following reviews: Harper and deMuelenaere, 1963; Morrison and Narayana Rao, 1966; Mauron, 1972; McLaughlan, 1972; Morrison, 1972). Measurements such as biological value, gross protein value, net protein utilization and protein efficiency ratio reflect changes in availability only when these have occurred in the limiting amino acid and give no information on the other nonlimiting essential amino acids present.

For studying changes in availability of amino acids brought about by cooking methods or processing conditions, or for testing the availability of amino acids in new protein foods such as textured vegetable proteins, a method is needed in which changes may be detected in the availability of each individual amino acid. An approach which could be used in determining specifically which amino acids have been affected would be of significant value.

In attempting to develop such a method, we devised diets in which amino acids would be present at "critical" levels. The critical level is defined as that level of each amino acid which would maintain a moderately rapid rate of growth but which, when reduced by a known amount (such as 20%), would result in significantly lower weight gains, PER (Protein Efficiency Ratio), and food efficiencies.

Our initial studies were intended to establish, where possible, critical levels (as defined above) for each essential amino acid (including tyrosine and cystine) by using casein plus L-amino acids. Studies were then conducted to determine whether changes in bioavailability could be detected in diets containing unheated and heated lactalbumin using the critical levels previously established in the experiments in which the casein-amino acid mixtures were fed.

### EXPERIMENTAL

ALL DIETS were analyzed for nitrogen by a macro-Kjeldahl method, and actual nitrogen content was used for calculating PER. Glutamine was added as needed to maintain the nitrogen content at a level equivalent to 10% protein ( $6.25 \times N$ ). Glutamine was selected instead of another nitrogen source because in studies comparing the utilization of  $\alpha$ -amino and amide nitrogen it was found that rats fed ad libitum ate more of a diet supplemented with glutamine than of one containing isonitrogenous amounts of glutamic acid (Womack and Wilson, 1969). Besides amino acids, protein, glutamine and  $\text{NaHCO}_3$  (in amounts required to neutralize the HCl of any added arginine, histidine or lysine), the diets contained vitamin mixture (General Biochemicals, Chagrin Falls, Ohio) 1%; salt mixture (Jones and Foster, 1942) 4%; nonnutritive fiber 4%; corn oil 10%; and cornstarch (to 100%).

Amino acid analyses of casein were done as previously described (Ahrens et al., 1966). For amino acid analyses of unheated lactalbumin, 200 mg samples were hydrolyzed for 22 hr at 110°C in 40 ml of 6N HCl (Bodwell et al., 1971). For tryptophan analyses, 16-hr hydrolyses at 110°C were done by using approximately 3.1M  $\text{Ba(OH)}_2$  (Knox et al., 1970). For both the acid and base hydrolyzates, duplicate or triplicate analyses were done on each of two hydrolyzates by using a Technicon amino acid analyzer.

Experiments with casein-free amino acid combinations

In these experiments, diets containing L-

amino acids (N.R.C. grade) and 5% casein were fed ad libitum for 2 wk to young male rats (Sprague Dawley strain from Charles River Breeding Laboratories, Wilmington, Mass.). The content of essential amino acids (plus cystine and tyrosine) in the control diets used in Experiments 1, 2 and 3 are shown in Table 1.

In Experiment 1, the control diets (including protein-derived and added free L-amino acids) contained each of the essential amino acids at the levels previously reported to be required for maximum growth of the rat (by Ranhotra and Johnson, 1965, for arginine; by Stockland et al., 1970, for lysine; and by Rama Rao et al., 1959, for the other amino acids). Separate experimental diets were fed in which each individual essential amino acid, in turn, was reduced 20%.

In Experiments 2 and 3, the essential amino acid levels of the control diets were reduced when necessary in order to establish critical levels not established in Experiment 1. As in Experiment 1, test diets were fed which contained levels of essential amino acids 20% lower than in the control diets.

Experiments with lactalbumin

For heat treatment, 2-kg batches of lactalbumin (11.31% N) were placed in shallow 12- $\times$ -18-in. aluminum pans and autoclaved uncovered for 30 min at 120°C with a subsequent fast exhaust and dry cycle.

In the first experiment, the unheated and the autoclaved lactalbumin were incorporated into diets at a level to furnish 10% protein (13.1% of the diet) and were fed for 3 wk to young male rats to determine PER. In a second experiment, diets equivalent to 10% protein

Table 1—Dietary levels (%) of essential amino acids<sup>a</sup> in casein-amino acid control diets

Amino acids	Experiment 1 % of diet	Experiment 2 % of diet	Experiment 3 % of diet
Arginine	0.41	0.30	0.166 <sup>b</sup>
Cystine	0.34	0.25	0.20
Histidine	0.20	0.20	0.16
Isoleucine	0.55	0.40	0.32
Leucine	0.70	0.50	0.50
Lysine	0.60	0.60	0.60
Methionine	0.16	0.16	0.16
Phenylalanine	0.42	0.30	0.30
Threonine	0.50	0.35	0.35
Tryptophan	0.11	0.11	0.11
Tyrosine	0.30	0.235 <sup>b</sup>	0.235 <sup>b</sup>
Valine	0.55	0.40	0.40

<sup>a</sup> Plus cystine and tyrosine

<sup>b</sup> All from casein



containing 6.5% of the unheated or autoclaved lactalbumin were supplemented with amounts of amino acids which would supply the critical levels without the contribution from the protein.

Finally, experiments were also carried out in which feeding trials were made using a series of test diets to detect possible heat damage to the individual amino acids. The control diets contained 6.5% autoclaved lactalbumin plus free amino acids, as above. In each test diet, except as noted below, protein-derived plus free amino acids were adjusted to be equal to the critical level added to the control diet. Table 2 shows the amounts of added free amino acids and the amounts contributed by the 6.5% lactalbumin for the control diet. Also included, as an exam-

ple, are the amounts of amino acids added when the availability of isoleucine was tested. The levels of amino acids were the same as in the control diet except for isoleucine. Added isoleucine (0.073%) plus protein-derived isoleucine (0.247%) was equal to the critical level (0.32%). Similar adjustments were made for each individual amino acid when testing for changes in the availability of that amino acid. In diets used to test the availability of arginine, leucine, tryptophan and tyrosine, all of the amino acid was supplied by the protein and none was added.

## RESULTS

IN TABLE 3 (Experiment 1) are shown

Table 2—Essential amino acids<sup>a</sup> in control and isoleucine test diets containing autoclaved lactalbumin

	Control diet		Isoleucine test diet Added amino acid % of diet
	Added free amino acid <sup>b</sup> % of diet	Protein-derived amino acid % of diet	
Arginine	—	0.143	—
Cystine	0.20	0.162	0.20
Histidine	0.16	0.191	0.16
Isoleucine	0.32	0.247	0.073 <sup>c</sup>
Leucine	0.50	0.611	0.50
Lysine	0.60	0.487	0.60
Methionine	0.16	0.104	0.16
Phenylalanine	0.30	0.175	0.30
Threonine	0.35	0.253	0.35
Tryptophan	0.11	0.110	0.11
Tyrosine	—	0.169	—
Valine	0.40	0.247	0.40

<sup>a</sup> Plus cystine and tyrosine

<sup>b</sup> Critical level (see text for definition) except for cystine, for which a critical level was not established

<sup>c</sup> Protein amino acid plus added free amino acid equals the critical level.

Table 3—Influence of a 20% reduction of individual dietary amino acids, from values shown in Table 1, on weight gains, food efficiency and protein efficiency ratios (PER) in Exp. 1

	No. rats	Weight gains <sup>a</sup> (g)	Food efficiency	PER
			Gain/g food (g)	
Casein control 1 <sup>b</sup>	10	70.4 ± 3.21 <sup>c</sup>	0.43 ± 0.008 <sup>c</sup>	4.55 ± 0.09 <sup>c</sup>
Arginine	10	70.5 ± 4.05	0.43 ± 0.011	4.65 ± 0.11
Cystine	9	71.4 ± 3.25	0.42 ± 0.009	4.46 ± 0.10
Histidine	10	51.3 ± 3.07 <sup>d</sup>	0.38 ± 0.013 <sup>d</sup>	4.00 ± 0.12 <sup>d</sup>
Isoleucine	10	64.4 ± 3.76	0.42 ± 0.008	4.44 ± 0.09
Leucine	8	63.4 ± 4.42	0.41 ± 0.012	4.36 ± 0.13
Lysine	9	67.0 ± 4.24	0.38 ± 0.008 <sup>d</sup>	4.04 ± 0.09 <sup>d</sup>
Methionine	10	37.6 ± 2.74 <sup>d</sup>	0.33 ± 0.008 <sup>d</sup>	3.56 ± 0.08 <sup>d</sup>
Phenylalanine	9	71.5 ± 5.11	0.42 ± 0.011	4.34 ± 0.12
Threonine	10	78.9 ± 4.14	0.44 ± 0.007	4.51 ± 0.08
Tryptophan	10	71.1 ± 4.45	0.40 ± 0.009 <sup>d</sup>	4.15 ± 0.09 <sup>d</sup>
Tyrosine	10	74.8 ± 3.41	0.43 ± 0.006	4.44 ± 0.07
Valine	10	72.8 ± 3.26	0.42 ± 0.008	4.40 ± 0.09

<sup>a</sup> In 14 days. Average initial weights of the groups were 57–59g.

<sup>b</sup> Added free amino acids plus casein amino acids at the levels shown in Table 1, Exp. 1

<sup>c</sup> SEM

<sup>d</sup> Significantly different from casein control group 1

weight gains, food efficiencies and PER for groups of animals fed the control diet, containing casein plus L-amino acids, or similar diets in which one amino acid at a time was reduced by 20%. The omission of all added methionine from the diet amounted to only a 17.5% reduction. For the histidine and methionine tests there were significant reductions from values for rats fed the control diet for all three parameters listed. For lysine and tryptophan, food efficiency and PER, but not weight gains, were significantly reduced from values for the control group. Food efficiencies would give the same results as PER in all cases if all of the diets contained exactly 1.6% nitrogen (equivalent to 10% protein). There is one case (see Table 4) where the nitrogen content (by analysis) of the diet deviated sufficiently from 1.6% (the calculated value) so that the value for food efficiency is significantly different from the value for the control group, while that for PER is not. For this reason all three parameters were used in evaluating results.

For Experiment 2, levels of histidine, lysine, methionine and tryptophan in the control diet (casein plus L-amino acids) were as in Experiment 1 and lower levels were selected for the other amino acids (see Table 1 for these values). Table 4 shows weight gains, food efficiencies and PER for rats fed the control diet or diets in which one amino acid at a time was reduced as before. In this experiment there were significant reductions from values for the control group in some or all of the parameters for groups in which leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine were reduced. The 20% reduction in histidine levels from the amount in the control diet did not, as it had in Experiment 1, have any significant effect on the parameters, nor did reductions in cystine or isoleucine. No test was made with tyrosine in this experiment because there was no added tyrosine in the control diet; thus the level could not be reduced.

A further revision of the dietary amino acid levels was made for Experiment 3 (Table 1). Reductions of 20% from these levels (Table 5) resulted in significant differences in weight gains, food efficiencies or PER from values for the control for all groups except those in which the test amino acid was cystine and, surprisingly, tryptophan. For Experiments 1 and 2 a reduction in tryptophan level from 0.11% to 0.088% had resulted in significant reductions in the parameters measured (except for weight gain in Experiment 1).

Table 6 shows the results of tests to study the susceptibility of individual amino acids of lactalbumin to damage by autoclaving. It can be seen that histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine were affected by autoclaving at

120°C for 30 min. Even for histidine and phenylalanine, where only 57–58% of dietary amino acid was derived from the protein, and for leucine where the protein supplied 122% of the critical level, there were significant differences from values for the control group for PER. Although data are not presented, similarly significant differences were also observed for weight gains and food efficiencies.

## DISCUSSION

### Critical levels

Critical levels suitable for determining changes in availability of nine of the essential amino acids were established under our conditions. The situation in regard to arginine, cystine and tyrosine is unresolved. For the present we can say that the critical level for arginine is less

than 0.166% and for tyrosine less than 0.235% (Table 4) when the diet contains 0.30% phenylalanine. In one experiment where amino acids were the only source of nitrogen, omitting arginine reduced weight gains 17%, food efficiency 14% and PER 15%. It is, of course, well known that the rat can synthesize arginine but not in quantities to support rapid growth. When tyrosine was omitted, weight gains were reduced 78%, food efficiency and PER 64%. Apparently enough of the limited quantity of phenylalanine present in these diets was converted to tyrosine to allow for slow growth. It is well known that phenylalanine can replace tyrosine totally if present in sufficient quantity (Womack and Rose, 1946).

A reduction of cystine to 0.20% had no effect (Table 5). In another study in which diets were fed containing 0.16 and

0.14% cystine, PER was significantly reduced from values found for the control group fed 0.20% cystine, but weight gains and food efficiency were not. Thus the matter of a critical level for cystine also needs additional study.

The tryptophan level may need to be reduced slightly. In Experiment 1, reduction to 0.088 did not significantly affect growth rate, but there was a significant decrease in food efficiency. In Experiment 2, all three parameters for the group fed the diet in which tryptophan was reduced were significantly different from control values.

### Lactalbumin

In the present study autoclaving decreased PER 43%, from  $3.45 \pm 0.09$  to  $1.98 \pm 0.10$ . Addition of the amounts of essential amino acids shown in Table 2 to a diet containing 6.5% autoclaved lactalbumin restored PER to  $3.30 \pm 0.12$ . The PER of unheated lactalbumin similarly supplemented was  $3.12 \pm 0.14$ . These data show that an imbalance was not created by the addition of these amounts of amino acids. Likewise, the autoclaved material contained no substances which would affect food intake and growth rate.

It has been known for many years that amino acids other than lysine are damaged when lactalbumin is autoclaved. As early as 1949, Davis et al. reported that the growth rate of young male rats was not increased by addition of lysine to a diet containing lactalbumin autoclaved for 60 min at 15 lb pressure. The data in Table 6 indicate that nine amino acids were damaged. Little or no effect of autoclaving on arginine and tyrosine was observed, even though the amounts in the diet from autoclaved lactalbumin must be close to critical levels. It is hard to believe that cystine is not affected by the heat treatment, particularly in view of the report of Iriarte and Barnes (1966) that autoclaving whole soybean flour at 120°C for 2 hr reduced cystine content from 1.56 to 1.15g/16g nitrogen. As stated earlier, however, the situation in regard to a critical level for cystine needs further study.

Since nine of the amino acids in the heated lactalbumin showed decreases in availability, the question can be raised as to whether decreases in availability or destructive losses of the amino acids are being measured. In either case, of course, the amino acids are lost nutritionally from the heated product. However, amino acid analyses of the heated lactalbumin showed that over 95% of the isoleucine, methionine, phenylalanine and tryptophan in the unheated lactalbumin was present in the heated lactalbumin (Womack et al., 1973).

### Application

For applications to other food pro-

Table 4—Influence of a 20% reduction of individual dietary amino acids, from values shown in Table 1, on weight gains, food efficiency and protein efficiency ratios (PER) in Exp. 2

	No. rats	Weight gains <sup>a</sup> (g)	Food efficiency Gain/g food (g)	PER
Casein control 2 <sup>b</sup>	12	74.3 ± 3.75 <sup>c</sup>	0.40 ± 0.013 <sup>c</sup>	4.23 ± 0.14 <sup>c</sup>
Cystine	11	68.8 ± 4.31	0.39 ± 0.009	4.04 ± 0.10
Histidine	8	66.0 ± 4.64	0.39 ± 0.013	4.06 ± 0.14
Isoleucine	10	76.7 ± 3.44	0.42 ± 0.007	4.55 ± 0.07
Leucine	12	23.6 ± 3.09 <sup>d</sup>	0.22 ± 0.025 <sup>d</sup>	2.23 ± 0.26 <sup>d</sup>
Lysine	12	62.1 ± 2.73 <sup>d</sup>	0.36 ± 0.009 <sup>d</sup>	3.97 ± 0.10
Methionine	12	41.9 ± 3.02 <sup>d</sup>	0.32 ± 0.012 <sup>d</sup>	3.22 ± 0.12 <sup>d</sup>
Phenylalanine	11	35.7 ± 2.18 <sup>d</sup>	0.29 ± 0.010 <sup>d</sup>	3.12 ± 0.10 <sup>d</sup>
Threonine	12	63.9 ± 3.70	0.35 ± 0.009 <sup>d</sup>	3.76 ± 0.09 <sup>d</sup>
Tryptophan	11	61.9 ± 3.52 <sup>d</sup>	0.35 ± 0.014 <sup>d</sup>	3.73 ± 0.15 <sup>d</sup>
Valine	11	54.4 ± 4.39 <sup>d</sup>	0.35 ± 0.018 <sup>d</sup>	3.57 ± 0.19 <sup>d</sup>

<sup>a</sup> In 14 days. Average initial weights of the groups were 60–61g.

<sup>b</sup> Added amino acid plus casein amino acids at the levels shown in Table 1, Experiment 2

<sup>c</sup> SEM

<sup>d</sup> Significantly different from casein control group 2

Table 5—Influence of a 20% reduction of individual dietary amino acids, from values shown in Table 1, on weight gains, food efficiency and protein efficiency ratios (PER) in Exp. 3

	No. rats	Weight gains <sup>a</sup> (g)	Food efficiency Gain/g food (g)	PER
Casein control 3 <sup>b</sup>	12	63.6 ± 3.60 <sup>c</sup>	0.38 ± 0.011 <sup>c</sup>	3.91 ± 0.11 <sup>c</sup>
Cystine	11	60.2 ± 3.72	0.37 ± 0.012	3.86 ± 0.13
Histidine	10	30.5 ± 4.20 <sup>d</sup>	0.25 ± 0.020 <sup>d</sup>	2.55 ± 0.20 <sup>d</sup>
Isoleucine	12	18.7 ± 4.36 <sup>d</sup>	0.17 ± 0.025 <sup>d</sup>	1.78 ± 0.26 <sup>d</sup>
Leucine	12	28.7 ± 1.92 <sup>d</sup>	0.25 ± 0.010 <sup>d</sup>	2.54 ± 0.10 <sup>d</sup>
Lysine	12	45.5 ± 4.25 <sup>d</sup>	0.33 ± 0.015 <sup>d</sup>	3.40 ± 0.16 <sup>d</sup>
Methionine	12	33.0 ± 3.04 <sup>d</sup>	0.27 ± 0.016 <sup>d</sup>	2.78 ± 0.16 <sup>d</sup>
Phenylalanine	11	27.6 ± 3.25 <sup>d</sup>	0.25 ± 0.020 <sup>d</sup>	2.62 ± 0.21 <sup>d</sup>
Threonine	12	41.2 ± 4.27 <sup>d</sup>	0.28 ± 0.015 <sup>d</sup>	2.77 ± 0.14 <sup>d</sup>
Tryptophan	12	65.4 ± 4.12	0.39 ± 0.012	4.01 ± 0.13
Valine	11	36.7 ± 4.44 <sup>d</sup>	0.29 ± 0.019 <sup>d</sup>	2.92 ± 0.19 <sup>d</sup>

<sup>a</sup> In 14 days. Average initial weights of the groups were 61–62g.

<sup>b</sup> Added amino acids plus casein amino acids at the levels shown in Table 1, Experiment 3

<sup>c</sup> SEM

<sup>d</sup> Significantly different from casein control group 3

Table 6—Decreases in Protein Efficiency Ratio (PER) when individual amino acids of autoclaved lactalbumin were tested for heat damage

Test amino acid	Protein amino acids		PER % of control <sup>a</sup>
	% in diet	% of critical level	
Arginine	0.143 <sup>b</sup>	c	112
Cystine	0.162	c	93
Histidine	0.091	57	62 <sup>d</sup>
Isoleucine	0.247	77	51 <sup>d</sup>
Leucine	0.611	122	51 <sup>d</sup>
Lysine	0.487	81	64 <sup>d</sup>
Methionine	0.104	65	68 <sup>d</sup>
Phenylalanine	0.175	58	81 <sup>d</sup>
Threonine	0.253	72	35 <sup>d</sup>
Tryptophan	0.110	100	68 <sup>d</sup>
Tyrosine	0.169 <sup>b</sup>	c	104
Valine	0.247	62	74 <sup>d</sup>

<sup>a</sup> Control diets contained the amounts of amino acids shown in Table 2. Each individual test diet contained the amounts of the amino acids in the control diet except for the amino acid under test. For that test amino acid the diet contained the amount from the protein shown in column 1 plus enough added amino acid so that the total was equal to the critical level.

<sup>b</sup> Total amount of the amino acid in the diet

<sup>c</sup> Critical level not established; 81% of dietary cystine was supplied by lactalbumin.

<sup>d</sup> Significantly different from control

teins or products, two different approaches can be used. In the case where bulk quantities of a product have been treated and untreated material is also available, an initial feeding trial can be made in which the protein-derived plus added free amino acids are equal to the critical level for both the treated and untreated product. If differences are observed, then one or more of the essential amino acids has been significantly affected. A second test, which includes a series of assays, can then be done to determine which amino acids have been affected. In this series, all amino acids would be added in amounts equal to the critical level with the exception of one essential amino acid, in turn; for that amino acid the amount from the protein

plus added free amino acid would equal the critical level.

In another application, a single food can be evaluated without comparisons with untreated (or differently treated) samples of the same product. In this case, a series of tests can be made which are analogous to the series in the second test above. Thus, each specific amino acid which has been affected can be identified.

Some proteins or foods contain such a low level of one or more amino acids that it is impossible to make diets in which an appreciable amount of the critical level would be supplied by the protein. However, if an amino acid were present at such low levels, the protein preparation or food would be of little value as a source for that particular amino acid.

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## INFLUENCE OF MOIST-HEAT TREATMENTS OF PEANUTS ON PEANUT PASTE CHARACTERISTICS

### INTRODUCTION

PEANUTS are traditionally processed into a variety of food products by means of dry heat processes such as oven roasting or cooking in hot oil. These roasting methods result in desirable quality changes such as moisture reduction, browning, textural improvement and development of typical roasted peanut flavor.

Processing peanuts with hot water or steam, though not as common as dry heat processing, has been found to result in products which are lacking in typical peanut flavor but have potential applications in a variety of food products such as meat analogs, beverages and confections. Mitchell (1950) prepared stable aqueous emulsions of varying consistencies by intensely agitating mixtures of finely ground peanuts with water; emulsion stability was increased by heating at temperatures of 150–190°F. Mitchell and Malphrus (1968) produced bland-flavored peanut flakes for use as a food ingredient by heating finely ground peanuts with water at 230–240°F for 20 min followed by drum drying. Moist heat processing has been employed in India to produce peanut milk (Miltone) free of the characteristic raw peanut flavor (Chandrasekhara et al., 1971). The acceptability of this product depended upon treatment with live steam to control the color and eliminate the natural peanut flavor.

Effects of moist versus dry heat on the chemical and physical characteristics of peanut proteins have been investigated. Pickett (1943) found that protein denaturation occurred much more rapidly with steam at 250°F than with dry heat at the same temperature. More recent studies by Neucere (1972), Neucere et al. (1972) and Thomas and Neucere (1973) have shown moisture and temperature effects on amino acid composition, nutritional value, and enzyme activity.

At present, however, little is known about the effect of moist-heat treatment of peanuts on the functional and organoleptic characteristics of peanut products. According to Mattil (1971), some empirical functionality tests of protein-containing materials could yield misleading information inasmuch as they often ignore the environmental reactions to which pro-

teins are exposed in food systems. The purpose of this investigation was to provide further information on the effects of varying hot water treatments of peanut kernels on moisture uptake, grinding and paste characteristics and paste quality for confectionery use.

### MATERIALS & METHODS

PEANUTS used in this investigation were of the Florunner variety (commercial U.S. No. 1 grade) which had been stored at 33°F and 65% relative humidity (RH) for 9 months (ca 7% moisture). The nuts were conditioned for skin removal by holding them at 85°F and 70% RH for 64 hr. The skins were removed by conventional mechanical means, and kernels having minor defects were removed by hand.

A 5 × 6 factorial design was utilized in which the kernels were heated in water at 90, 120, 150, 180 and 210°F in a steam-jacketed kettle for 15, 30, 45, 60, 75 and 90 min with two replications of each treatment. The treatments selected for the study were based upon preliminary investigations of the effects of steam and hot water on the grinding characteristics and quality of paste produced from Florunner peanuts. After treating, draining, and cooling, samples were held overnight in closed jars at 75°F since preliminary investigations had shown that the treated samples had to reach equilibrium before the grinding operation could be performed satisfactorily. They were ground to pastes the following day in a pilot-scale stone mill (Morehouse-Cowles, Inc., Los Angeles, CA). In order to obtain a fine grind, the mill was set at a stone clearance of 0.004 in. The nuts were ground twice to attain a uniform distribution of moisture throughout each sample.

Moisture content, shear resistance, color, grinding characteristics and subjective flavor ratings of the paste were measured. Since it was desirable to evaluate the potential application of peanut paste in a finished product, macaroon cookies were selected as an index to its performance in a food system. Macaroon cookies utilize almond paste as a major ingredient, and because of similarities in consistency and other qualities, peanut paste could easily be substituted for almond paste in the cookie formula. Sensory qualities of the baked cookies were determined.

#### Moisture

Moisture content was determined in duplicate as loss of weight from 5-g samples of ground peanuts after heating for 24 hr at 158°F under a vacuum of 29 in. Hg.

#### Shear resistance

Shear resistance was determined on duplicate 60-g samples of the peanut paste. Each

sample was pressed into a 4-oz wax-coated paper cup so that it conformed to the shape of the cup. The paste cake was then removed from the cup and transferred to a Food Technology Corp. Shear Press, Model TP-1, for texture measurement. A universal cell equipped with an extrusion grid base and close tolerance piston formed the extrusion test cell. A 300-lb transducer ring, down-stroke speed of 30 sec, and recorder range setting of 50 were used. Data were reported as kg force per g peanut paste.

#### Color

Following texture determinations, the paste cake was transferred to a Gardner Color Difference Meter, Model C-4, for color measurements. A white chromatic reflectance standard (L = 89.3, a = -0.9, b = -0.9) was used to standardize the instrument. Readings were taken of the L, a, and b values, and the a/b ratios were calculated.

#### Sensory evaluation

A panel of five judges, experienced in peanut flavor perception, evaluated the flavor of the peanut paste using a nine-point hedonic scale ranging from 9 (excellent) to 1 (extremely poor). Macaroon cookies were prepared according to the following recipe (Anon., undated)

Ingredients	Weight (g)
Almond paste	56.5
Egg white	17.0
Sugar, powdered	22.5
Sugar, granulated	28.5

substituting nonsweetened peanut paste for almond paste. All samples were baked at 350°F for 15 min. and comparative evaluations for appearance, color, aroma, texture and flavor were performed.

Data presented in the tables and figures were subjected to statistical analysis of variance. Significance of mean differences was determined by the Duncan's multiple range test and are shown on the tables only.

## RESULTS & DISCUSSION

### Observations in preliminary trials

Preliminary tests showed that unheated peanuts could be ground to a thick, creamy paste-like product but exhibited poor baking properties. Cookies prepared from this paste were thin and flat, had a tough texture and pronounced raw peanut flavor. Also, the natural peanut oil separated from the paste upon the addition of water. Peanut kernels that were pressure cooked with live steam at 230°F for 45 min could be ground satisfactorily without an equilibration period

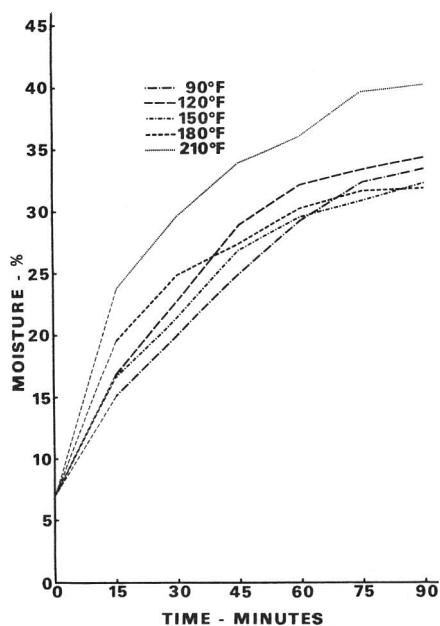


Fig. 1—Effect of heating time and temperature on the moisture content of peanuts heated in water.

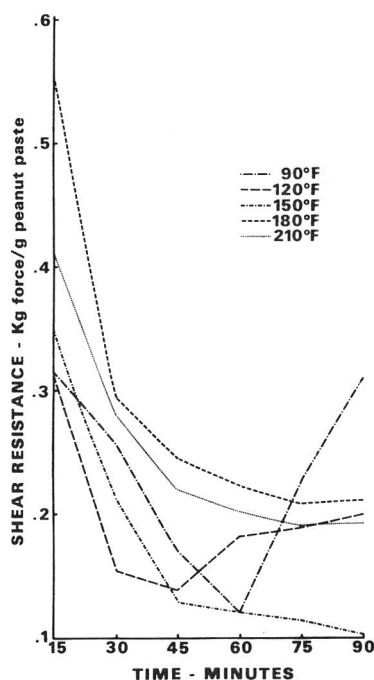


Fig. 2—Effect of heating time and temperature on the shear resistance of peanuts heated in water.

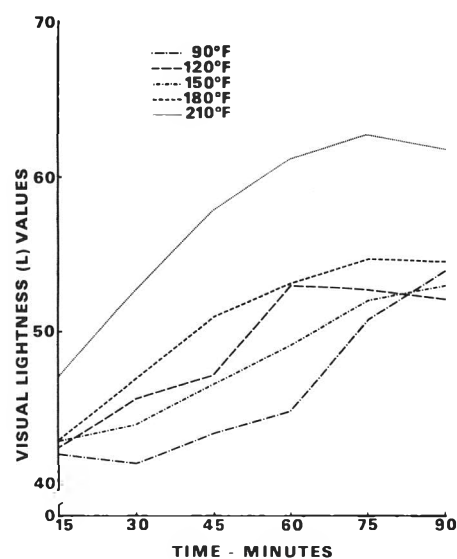


Fig. 3—Effect of heating time and temperature on the visual lightness (L) values of peanuts heated in water.

but the product was dry, crumbly and undesirably dark.

Peanut kernels soaked overnight in water at room temperature, cooked in live steam as above and then ground, produced a mild-flavored, thick, creamy white paste. Macaroon cookies baked from this high-moisture paste had a very desirable tender texture. Kernels cooked with live steam at a lower temperature (210°F) for 30 and 60 min could not be ground without overnight equilibration. The baked products from this paste were extremely dry and tough.

Kernels immersed in water at room temperature for 5 min could not be ground even after overnight equilibration. However, kernels heated in water at 210°F for the same period could be ground readily after equilibration and produced a thick, creamy paste with desirable baking properties. Cookie batter prepared from this paste was stiff and somewhat lumpy but produced attractive cookies with good volume and flavor. When the time of heating at 210°F was extended to 60, 90 and 120 min, no equilibration was needed before grinding to prepare attractive paste products. Cookies prepared from these pastes retained their shape during baking, had very tender texture, and no pronounced raw peanut flavor.

#### Moisture

Statistical analysis of the data in Fig-

ure 1 showed highly significant ( $P < 0.01$ ) effects on moisture absorption with temperature and time. Moisture increased significantly for each 15-min interval except from 75 to 90 min at 120, 180 and 210°F. The greatest moisture absorption occurred at 210°F. At temperatures between 120° and 210°F, however, trends in moisture absorption were not completely clear. The moisture uptake of nuts heated at 150 and 180°F for 45 min or longer was lower than for those heated at 120°F. While the variation in moisture uptake may be influenced by a number of factors including the response of the protein, lipid, carbohydrate and enzymes to heat and water, the most plausible explanation could be that there is a sealing off of the outer layer of the kernels at the intermediate temperature treatments. The authors have previously noted (unpublished data) that, in attempts to inactivate the enzymes of peanuts by immersing them briefly in hot water, an impervious outer layer is formed on the kernel which markedly reduced subsequent moisture exchange.

#### Shear resistance

The shear resistance values of peanut pastes were significantly affected by temperature, time, and temperature  $\times$  time interaction. At 150, 180 and 210°F, resistance to shear decreased as heating time increased (Fig. 2). At 120°F, shear resistance values decreased as heating

time increased until 45 min when a reverse trend occurred. A similar pattern, though more extreme and occurring after 60 min, was noted at 90°F. The unusual patterns at the intermediate temperatures are consistent with those for water uptake. According to Lumry (1973) protein uptake of water under such conditions may drive oily groups from the water phase causing the protein to form clusters characteristic of globular structures.

The shape of the shear resistance curves recorded by the shear press varied substantially in height, width and slope. As the kernel heating time at 150, 180 and 210°F was increased the curve height was reduced. The slope of the curve was also reduced indicating less shear resistance. Double peaks occurred more frequently with samples heated for 30 min or less indicating less homogeneity and more irregular shear resistance. Also, the excessive gumminess noted in samples with the lower times and temperatures of heating disappeared as the heating time and temperature increased. This is further evidence of the complex changes in functional characteristics that occur when the peanuts are subjected to varying time and temperature conditions of moist heat.

#### Color

Both time and temperature had highly significant effects ( $P < 0.01$ ) on the lightness (L) and yellowness (b) values, but not on the redness (a) or a/b ratios of

Table 1—Effect of heating time and temperature on the flavor scores of paste prepared from peanuts heated in water

Heating time (min)	Flavor scores <sup>a</sup>					Mean
	Temperature (° F)					
	90	120	150	180	210	
15	5.2	4.4	5.9	4.8	6.2	5.30
30	4.2	4.4	4.6	5.5	6.6	5.06
45	3.8	4.3	5.2	5.8	6.8	5.18
60	3.4	5.4	5.6	5.7	6.6	5.34
75	4.6	5.1	5.2	6.2	7.0	5.62
90	4.1	5.3	5.6	6.6	6.7	5.66
Mean	4.22d	4.82c	5.35b	5.77b	6.65a	

<sup>a</sup> Mean values having different letters are significantly different ( $P < 0.01$ ).

Table 2—Effect of heating time and temperature on the color scores of macaroon cookies prepared from peanuts heated in water

Heating time (min)	Color scores <sup>a</sup>					Mean
	Temperature (° F)					
	90	120	150	180	210	
15	5.9	7.1	6.1	7.1	7.7	6.78bc
30	6.7	6.8	7.4	7.7	7.8	7.28a
45	6.4	6.5	7.4	7.5	7.5	7.06ab
60	5.9	6.4	7.2	7.4	6.5	6.68bc
75	4.9	5.6	7.2	7.6	6.6	6.38cd
90	4.6	5.9	6.8	6.9	6.3	6.10d
Mean	5.73c	6.38b	7.02a	7.37a	7.07a	

<sup>a</sup> Mean values having different letters are significantly different ( $P < 0.01$ ).

paste samples from moist-heated peanuts. L and b values tended to increase as heating time and temperature increased. These results agreed with observations of increasing visual lightness with increasing heating time and temperature. The differences in L values (Fig. 3) also show an interaction of temperature and time. The erratic behavior of the values with increasing time of treatment at 120°F appears to be another manifestation of the unusual changes in paste properties that occur at this temperature. The higher L values at 180 and 210°F appear to be

related to conditions that support rapid inactivation of enzymes that may be involved in color changes of the paste.

#### Grinding characteristics

Kernels heated in water at 180 and 210°F were very easy to grind; the ground product was paste-like and creamy in consistency and was easy to handle since it was not sticky. Nuts heated for 15 min at all temperatures produced coarser pastes than those heated longer. Good grinding characteristics occurred also in peanuts heated at

90 and 150°F. Samples heated at 120°F for 75 and 90 min, however, were more difficult to grind with force feeding necessary on the second pass. While these samples provided a smooth paste after grinding, the product was both sticky and gummy. The problem encountered in grinding these samples appeared to be associated with the phenomena indicated in the water uptake and shear resistance data.

#### Sensory evaluation

Heating temperature had a highly significant effect on the flavor scores of peanut paste. Data in Table 1 show that the peanut flavor of the paste was improved as heating temperature increased. Comments by the panelists indicated that samples heated in water at the higher temperatures had less of the flavor usually associated with raw, wet peanuts and were not as "harsh" or "bitter" as those heated at lower temperatures. Baked macaroon cookies prepared from peanut paste are shown in Figure 4. Both heating time and temperature had highly significant effects on the appearance, color, aroma, texture and flavor of the cookies. In preparing the cookies, it was noted that the batters prepared from nuts heated for longer times were consistently smoother, thinner and required less shaping than those heated for shorter times. The samples heated for extended lengths of time tended to form large air bubbles and also spread more during baking—hence the gradation in sizes.

Peanut pastes from kernels heated for 15-min formed coarse, stiff batters and required considerable shaping when placed on the baking sheet. Since they contained less moisture than those heated for extended lengths of time, they did not spread during baking nor develop air bubbles. The lumpy nature of these batters resulted in the rather rough and slightly "pebbled" appearance of the baked product.

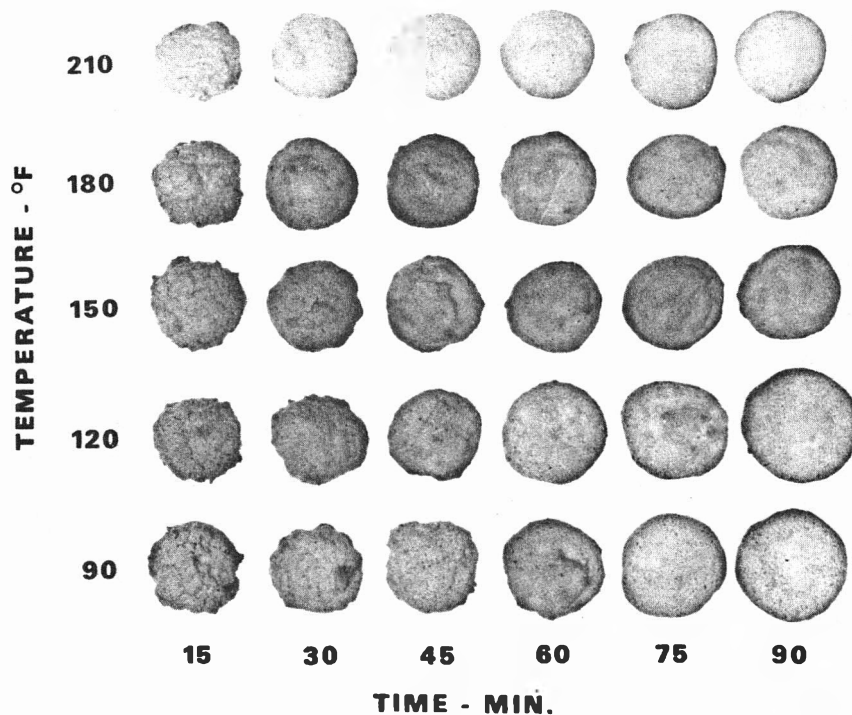


Fig. 4—Macaroon cookies prepared from peanut paste.

Table 3—Effect of heating time and temperature on the texture scores of macaroon cookies prepared from peanuts heated in water

Heating time (min)	Texture scores <sup>a</sup>					Mean
	Temperature (°F)					
	90	120	150	180	210	
15	5.9	6.2	6.2	7.0	6.6	6.38a
30	6.1	6.1	6.1	7.1	6.4	6.36a
45	5.6	6.0	5.9	7.2	6.8	6.30a
60	5.3	6.0	5.6	7.0	5.8	5.94ab
75	4.8	5.8	5.4	6.8	5.7	5.70b
90	4.8	5.9	5.6	6.4	5.3	5.60b
Mean	5.42c	6.00b	5.80bc	6.92a	6.10b	

<sup>a</sup> Mean values having different letters are significantly different ( $P < 0.01$ ).

Table 4—Effect of heating time and temperature on the flavor scores of macaroon cookies prepared from peanuts heated in water

Heating time (min)	Flavor scores <sup>a</sup>					Mean
	Temperature (°F)					
	90	120	150	180	210	
15	7.0	6.5	7.1	6.9	7.2	6.94a
30	6.1	5.4	6.4	6.8	7.2	6.38b
45	3.9	5.3	5.5	7.4	6.9	5.80c
60	2.9	6.0	5.4	7.1	6.1	5.50cd
75	5.2	5.8	5.5	6.2	5.6	5.66cd
90	4.9	5.3	5.3	5.6	5.1	5.24d
Mean	5.00c	5.72b	5.87b	6.67a	6.35a	

<sup>a</sup> Mean values having different letters are significantly different ( $P < 0.01$ ).

The sensory scores for color of macaroon cookies prepared from moist-heated peanuts appear in Table 2. There was a more pronounced development of brown color in cookies prepared from nuts heated for 45 min or less and was preferred by the panelists. More browning occurred in cookies prepared from nuts heated at 180°F than at other temperatures.

Texture scores appear in Table 3. The texture of cookies prepared from nuts heated for 60–90 min was described as “gummy, sticky and doughy” and was influenced by the high moisture content of the pastes. Moisture content of the paste ranged from 30–40% in samples heated for a prolonged period. The low-moisture pastes, on the other hand, contained 15–23% moisture and produced cookies with “tough, chewy” textures. The highest average texture score was obtained for samples that were moist-heated at 180°F for 45 min and contained 27% moisture.

Heating time and temperature both had highly significant effects on the flavor scores of macaroon cookies prepared from moist-heated peanuts. Since aroma and flavor scores were similar, only flavor scores are shown (Table 4). Panelists preferred the mild nut flavor of cookies prepared from nuts heated for 45 min or less, particularly at 180 and 210°F. The

flavor of cookies prepared from nuts heated at higher temperatures was described as “nutty and sweet.” Some off-flavors described by the panelists as rancid and stale were noted in samples prepared from nuts heated at 90°F for 45 and 60 min.

It is evident that the properties of the peanut paste and quality of baked cookies prepared from the paste were influenced by the time and temperature of moist heat treatment, and that these treatments also affected the moisture content of the paste and the baked cookies. Finished products having the highest overall quality were those from kernels heated in water at 180°F for 30–45 min and at 210°F for 15 min. The moisture content of pastes prepared from these treatments ranged from 23–27%.

These data suggest that the physical and organoleptic properties of peanuts are sensitive to moist heat treatment and that such treatments can have a substantial influence on their use in food systems. Based upon favorable functional, sensory and nutritional qualities, moist-heated peanuts may have increasing application in industry for meat substitute, beverage, confectionery and snack food products. The potential outlets for moist-heated peanut products also enhance the outlook for marketing peanuts other than as dry roasted items.

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## PREPARATION AND CHARACTERIZATION OF $\alpha$ -AMYLASE IMMOBILIZED ON COLLAGEN MEMBRANES

### INTRODUCTION

MORE THAN 3 billion pounds of glucose primarily for consumption by the food industry are produced annually (U.S. Dept. of Commerce, 1967) in the United States by the enzymatic conversion of starch, making this process the largest industrial process utilizing enzymes.

To produce glucose enzymatically, a starch suspension gelatinized by heating is pretreated with an  $\alpha$ -amylase preparation which partially degrades the starch to a form more susceptible to subsequently added fungal glucoamylase. According to Kooi and Armbruster (1967) conversion is complete usually in 72 hr and glucose yields of 97% are regularly obtained. Quantitative conversion is not reached, possibly because of the extended contact with glucoamylase which also catalyzes the polymerization of glucose to predominantly  $\alpha$ -(1-6) linked oligosaccharides. Furthermore, the enzyme preparations deteriorate and must be replenished during this period, and finally they must be separated from the product and discarded.

Immobilized enzymes have potential as catalysts in these reactions since they often tend to be more stable than the free enzyme, and can be recovered from the reaction mixture to be reused for long periods of time. Furthermore the product need not be contaminated with an extraneous protein because of the ease of removal of the insoluble catalyst. The use of immobilized amylases in a continuous flow system for the starch conversion process may also circumvent the problem of the back polymerization reaction, by decreasing residence time and contact with the enzyme.

Several reports have appeared in the literature which describe studies on the formation of glucose from starch by a continuous process in which starch solutions flow past amylase immobilized on inert matrices such as glass (Weetall and Havewala, 1972; Weetall, 1973) and DEAE-cellulose (Smiley, 1971). Recently Vieth et al. (1972) have reported on the

unusual properties and advantages of collagen as a support for enzymes. The present study was undertaken to prepare various amylases immobilized on membranes formed from collagen and to compare their properties with the free enzymes. In this paper are presented the findings of the various parameters that were studied to gain an understanding of the effects of immobilization of hog pancreatic  $\alpha$ -amylase (prepared from pancreatin) on collagen membranes.

### EXPERIMENTAL

THE MATRIX used for these studies was a membrane prepared from a dispersion of homogenized hide powder collagen either by codispersion or impregnation. Unless otherwise indicated, these studies were performed using the codispersed membrane.

#### Hide collagen membrane by codispersion

Crude hide collagen (100g) was dissolved in 400 ml of distilled water. The pH was lowered to 4.0 using lactic acid and the dispersion adjusted to the desired consistency. The dispersion was then blended and the large air bubbles removed by vacuum. At this point 35 mg of crystalline  $\alpha$ -amylase in buffer solution was added and the resulting collagen-enzyme complex stirred for 30 min to ensure uniform mixing of the enzyme in the collagen matrix. The pH of the resulting dispersion was checked and

if necessary adjusted to pH 4.5. A membrane (20 × 20 cm) was cast on a mylar support using a Gardner knife (Vieth et al., 1972).

#### Hide collagen membrane by impregnation

A 2 × 2 cm square of membrane prepared without adding enzyme was submersed in a solution of enzyme containing 10 mg of crystalline pancreatic  $\alpha$ -amylase per ml for 2 days at 0°C or at room temperature.

#### Preparation of hog pancreatic $\alpha$ -amylase

For these studies crystalline  $\alpha$ -amylase which had a specific activity of 750 I.U. per mg was used. The enzyme was prepared as described by Schramm and Loyer (1966) and modified by Romano (1968) from pancreatin powder (4 times NF strength) which was a gift from Takamine Laboratories, Clifton, N.J.

#### Assay of soluble amylase

**Manual procedure.** Amylase was assayed using sodium borohydride-treated starch which eliminated background reducing values, as previously described (Strumeyer, 1967).

**Automated procedure.** Routinely, the enzyme rate was continuously assayed at constant temperature, in a water-jacketed vessel at pH 7.0 (20 mM Tris-HCl). One unit of activity represents the production of 1  $\mu$ mole of dextrose equivalent per min at 30°C as measured by the ferricyanide procedure. Sodium borohydride-treated starch (Strumeyer, 1967) was used to minimize interference by the

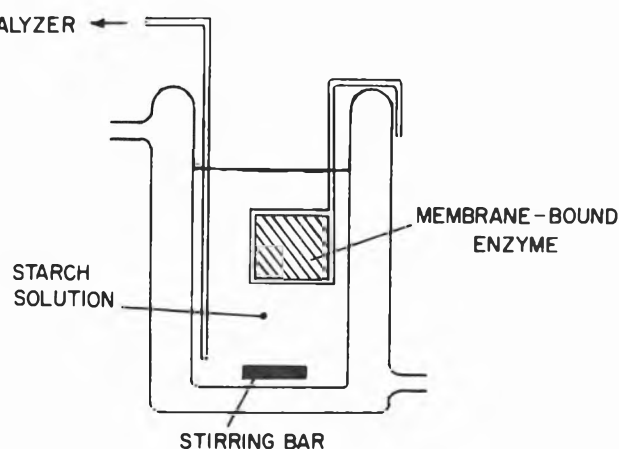


Fig. 1—Water-jacketed reaction vessel containing the collagen membrane supported on a frame, suspended in the constantly stirred reaction medium.

<sup>1</sup>Dept. of Biochemistry & Microbiology, College of Agriculture & Environmental Science  
<sup>2</sup>Dept. of Chemical & Biochemical Engineering, College of Engineering



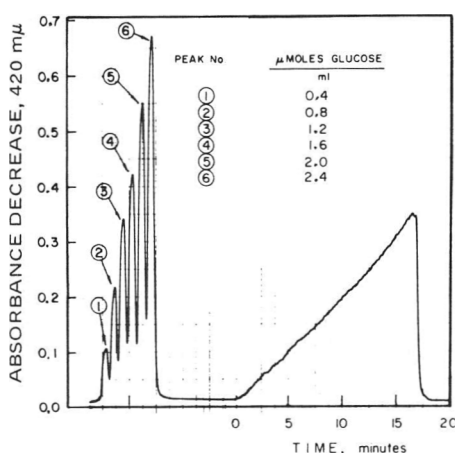


Fig. 2—Glucose standard curve obtained with Autoanalyzer at a rate of 60 samples per hour. Also, the rate of glucose reducing equivalents produced by the amylase membrane immersed in the reaction vessel containing starch.

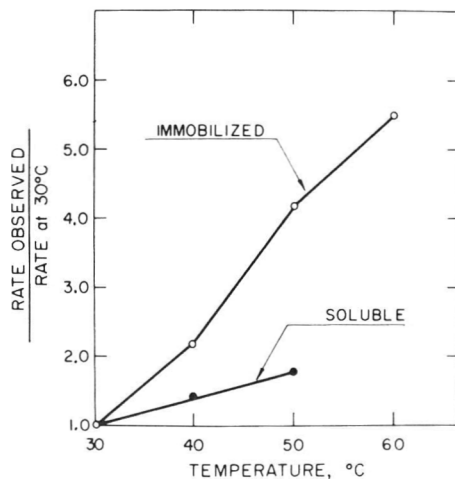


Fig. 3—Comparison of the effect of temperature on the rate of the hydrolysis of starch by the immobilized and soluble enzymes.

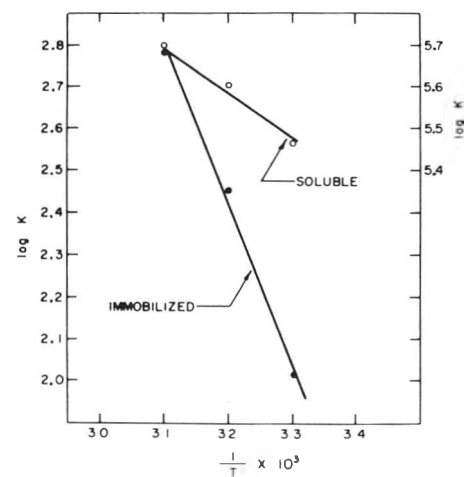


Fig. 4—Arrhenius plot for calculation of activation energy. Temperature is expressed as degrees Kelvin.

reducing end groups inherent in starch. 30 ml of a starch solution was added to the heated vessel and the solution was continually stirred with a magnetic bar until the desired temperature was reached. After temperature equilibration of the enzyme solution, an aliquot was added to the reaction vessel. A probe of stainless steel tubing connected to the Technicon pump via 0.025 inch (i.d.) tubing was immediately inserted into the vessel. The reaction mixture was stirred at a constant rate, aspirated and the dextrose reducing equivalents that formed were continuously monitored. The procedure described by Strumeyer and Romano (1966) was used except that a Gilford Model 300 Spectrophotometer and a model VOM-11 B & L Recorder replaced the corresponding Technicon units and the sampler was omitted.

**Assay of immobilized amylase**

The  $\alpha$ -amylase membrane was washed with buffer and exposed to a solution of 1% starch in 20 mM Tris-HCl buffer, pH 7.0 for 5 days before being assayed. A 2 x 2 cm square of membrane was glued to a frame on a rod (Fig. 1) and inserted into the reaction vessel. The dextrose equivalents produced were assayed by the automated procedure. Between uses, the enzyme membranes were stored in the 1% starch solution in the refrigerator.

**Determination of the effect of tanning**

Attempts were made to optimize the covalent linking of the enzyme to the membrane by tanning with various concentrations of glutaraldehyde for different lengths of time.

A 2 x 2 cm membrane was tanned by soaking in 10 ml of a 1-10% solution of glutaraldehyde. The excess glutaraldehyde and non-permanently fixed enzyme were removed by washing the enzyme impregnated membrane with running distilled water for 2 min. The membranes were air dried and stored in a 1% starch solution in the cold for several days prior to assay.

**Determination of the effect of pH**

The starch solutions were adjusted to the required pH by using 20 mM Tris buffer. The membrane was washed in buffer prior to use and blotted dry between each reaction. The enzyme dilutions as well as the starch solution used in the soluble enzyme assay were adjusted to the pH required.

**Determination of the effect of chloride ion concentration**

The starch solutions were made up to the desired concentration of chloride ion by adding NaCl. A range of 0.01-0.26M was used for the immobilized enzyme studies and a range of 0.01-0.36M was used for the soluble enzyme.

**Determination of the effect of temperature**

Temperature was maintained by circulating water from a constant temperature bath through the water-jacketed reaction vessel. The starch solution was equilibrated to the required temperature prior to inserting the membrane. The soluble enzyme sample and the starch solution were equilibrated separately in a constant temperature bath prior to starting the reaction by adding starch to the enzyme.

**Determination of the effect of storage**

Membranes of known reactivity were stored in the refrigerator in: (a) 20 mM Tris at pH 7.0

containing 3 mM CaCl<sub>2</sub> and 7 mM NaCl; (b) 1% starch made up in this buffer; or (c) distilled water, for 21 days. After this time, the membranes were removed and their rates determined.

**RESULTS**

THE INITIAL RATE for the formation of glucose reducing equivalents as determined with the automated procedure was linear during the period assayed (20 min) for both the soluble and immobilized  $\alpha$ -amylases. A typical rate profile obtained by aspirating the reaction medium and continuously monitoring the reducing groups formed by a membrane prepared by codispersion is shown in Figure 2. This assay procedure permitted the visualization of a continuous rate for the amyolytic process in contrast to the point assays ordinarily used for amylase assay. Rates were calculated from the decrease in absorbance and were expressed in terms of  $\mu$ moles of glucose equivalents formed per minute.

To determine the effect of different conditions on retention of activity so that membranes could be stored for repeated use,  $\alpha$ -amylase membranes were stored in

Table 1—Effect of storage conditions on activity

Membrane no.	Condition	Days	% Activity
8	20 mM Tris, 3mM CaCl <sub>2</sub> 7 mM NaCl, pH 7.0	21	19
2	1% Starch in above buffer	21	37
7	Distilled water	21	0

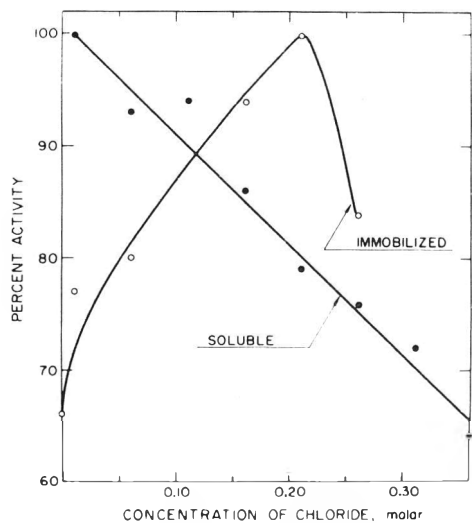


Fig. 5—Rate of starch hydrolysis as a function of chloride concentration.

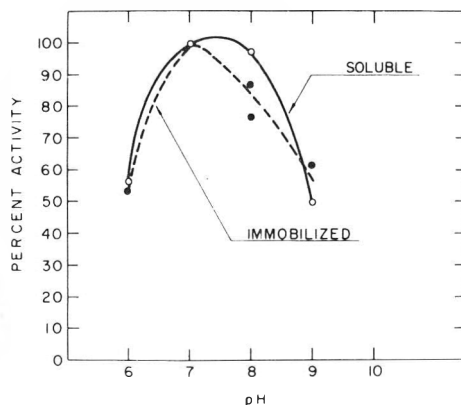


Fig. 6—Effect of pH on the hydrolysis of starch by free amylase and by the immobilized enzyme.

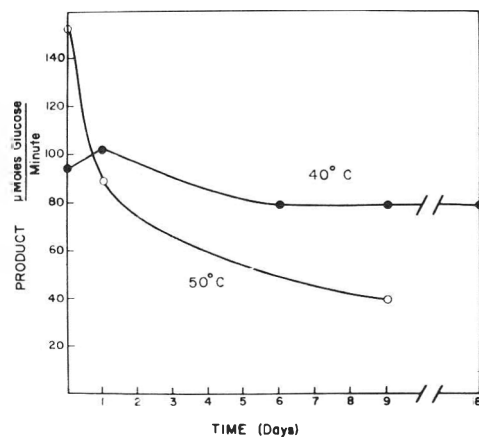


Fig. 7—Comparison of the initial rate of reaction of the immobilized enzyme after intermittent use at 50°C and at 40°C.

the refrigerator and their activity observed for a period of 3 wk. The data in Table 1 were collected using three different  $2 \times 2$  cm sections of membrane which were cut from the same codispersed membrane. It is apparent that activity is completely lost when the collagen-immobilized enzyme is stored in distilled water. On the other hand, an activity of approximately 37% of the original activity was maintained when the membrane was stored in the starch reaction mixture in the cold. Thereafter, the membranes were routinely stored in the starch solution after preparation and stabilization to minimize deterioration between experiments.

The effect of temperature on the rate of the reaction catalyzed by the soluble enzyme and on the rate of the immobilized enzyme were compared and the data obtained are presented in Figure 3. Although an acceleration in the rate of hydrolysis of starch was observed with the soluble  $\alpha$ -amylase as the temperature was raised, the increase in rate was less than twofold at 50°C when compared to 30°C. At 60°C the soluble enzyme was inactive. Control experiments showed that thermal inactivation occurred already at 50°C since when the enzyme was preincubated for 5 min at 50°C and assayed at 30°C, a 25% decrease in activity was noted. On the other hand, the initial rate for the immobilized enzyme increased 400% when the temperature was raised to 50°C, and increased 550% when assayed at 60°C.

The activation energy for these reactions was determined graphically as shown in Figure 4 and is 10.19 kcal/mole for the immobilized enzyme and 4.45 kcal for the soluble enzyme.

Since pancreatic  $\alpha$ -amylase has been reported to show a requirement for chloride ions for maximum activity, it was of interest to see if chloride was necessary for the expression of activity by the immobilized membrane. The effect of chloride concentration on the rate of starch hydrolysis is shown in Figure 5. Chloride (20 mM) is optimal for the soluble enzyme, but increased chloride levels inhibit activity. In contrast, the chloride requirement for the immobilized enzyme is increased 20-fold and a sharp maximum activity is observed at 0.22M chloride rather than at 0.01M. Higher chloride concentrations strongly inhibit the immobilized enzyme.

The free  $\alpha$ -amylase displays a broad pH optimum with essentially no change in activity between pH 7.0 and pH 8.0 as shown in Figure 6. On the other hand, the immobilized enzyme shows a pH optimum at pH 7.0 while at pH 8.0 there is only 80% activity compared to the soluble enzyme. On the acid side, however, the rate declines similar to the soluble enzyme.

The stability of codispersed membranes reacted intermittently at 40°C and 50°C is compared in Figure 7. Initially, the rate at 50°C is higher but the membrane deteriorates more rapidly than the membrane reacted at 40°C. Finally, the rate at 50°C stabilizes below the value for the membrane used at the lower temperature. It is not clear whether the enzyme is inactivated at the higher temperatures or if enzyme is being washed off the membrane faster at the higher temperature. Since less deterioration occurs at 40°C, and a greater activity is sustained at that temperature, 40°C was used as the standard temperature.

Interestingly, when an amylase membrane is assayed first at 40°C and then at 50°C, the activity observed when it is returned to 40°C is 110% higher than originally measured at that temperature. A similar effect was noted by Sheiman and Macmillan (personal communication) for pectin lyase similarly immobilized. The reason for this phenomenon is not understood. Perhaps the higher temperature causes a realignment of the reactive groups in the enzyme producing a more reactive configuration which is stabilized upon returning to the lower temperature. It may also be possible that additional sites are exposed at the higher temperature which are preserved when the temperature is lowered.

A study in which some of the parameters of the tanning system were examined in an attempt to arrive at optimum conditions for tanning the membrane is summarized in Table 2. This table is also a comparison of the effectiveness of immobilization of amylase by impregnation and codispersion. The results of these experiments show that exposure to 5% glutaraldehyde for less than 15 sec yields high activity membranes. Longer tanning times or glutaraldehyde concentrations of higher than 5% reduce or eliminate activity of the immobilized enzyme.

When the two immobilization techniques were compared and the membranes used tanned for 10 sec with 5% glutaraldehyde, the codispersed membrane (Table 2) at first showed a rate which was four times as great as the membrane prepared by impregnation. However, as pictured in Figure 8, after 9 days the codispersed membrane lost 90% of its initial activity, while after 21 days the impregnated membrane had not

changed. After 40 days of intermittent use, it lost 40% of its activity, but was still 2.4 times more active than the co-dispersed enzyme after only 9 days.

### DISCUSSION

THE ADVANTAGES INHERENT in the use of immobilized enzyme, e.g., reusability of the catalyst and elimination of contamination of the reaction mix with foreign materials have stimulated considerable interest in these derivatives as catalysts for industrial processes as well as tools for the research laboratory. The amylases are probably the most widely used enzyme in industry and have been thoroughly characterized. Consequently, an understanding of properties of immobilized amylases are of considerable practical importance. In this study, hog pancreatic amylase isolated from commercial pancreatin was chosen to serve as a model enzyme because we had it available in crystalline form and its properties had been studied in this laboratory.

We have employed two types of techniques for immobilizing amylase in collagen membranes: (1) *codispersion*, which involves immobilization by adding the enzyme to the collagen homogenate prior to casting the membrane, and (2) *impregnation*, which is used to form an enzyme-membrane, by soaking a preformed membrane in a solution of enzyme. In either case, it is subsequently possible to fix the enzyme to the membrane by cross-linking (tanning) with glutaraldehyde solution.

The membrane tanned in 1% glutaraldehyde had a high initial rate which appeared to increase after storage for 6 days. The increase may represent the release of soluble enzyme into the reaction medium from the deteriorating membrane or may be due to an increased accessibility to the reactive sites because of an increase in surface area. This type of membrane, however, could not be

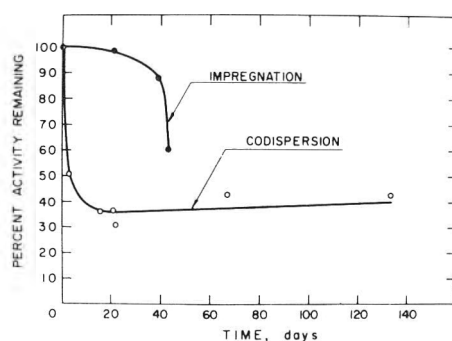


Fig. 8—Comparison of the rate of hydrolysis of starch by a codispersed and an impregnated membrane after intermittent use.

reused and was discarded because it lost its rigidity and began to disintegrate. Physical weakness is characteristic of membranes tanned at low levels, or not tanned at all.

High concentrations of glutaraldehyde (10%) formed sturdy and long-lasting membranes but were useful only when applied to a codispersed membrane, tanned for no more than 15 sec. When longer tanning times were used, the membranes had no activity. At 5% glutaraldehyde for 10 or 15 sec, one membrane retained 60% of its initial activity after intermittent use for 43 days. These observations were made on 2 × 2 cm squares of membranes. However, when a large membrane, 21 × 12 cm, was tanned in 10% glutaraldehyde for 30 sec, it exhibited activity whereas the same conditions produced a completely inactive membrane from a 2 × 2 cm square. Thus, size of membrane appears also to be a variable factor but was not evaluated in these studies.

These results demonstrate that either the codispersion or impregnation techniques for immobilization of α-amylase

produced active membranes which hydrolyzed starch. However, the impregnated membrane, which undoubtedly bound less enzyme than was distributed through the codispersed membrane, retained higher activity for a longer period of time than did the codispersed membrane. Also, since less enzyme was used for preparing the membrane by impregnation, this type of membrane warrants further attention.

The greater activity manifested by the impregnated membrane to which enzyme was adsorbed compared to the codispersed membrane with enzyme distributed throughout its mass may reflect the inaccessibility of the polymeric substrate beyond the surface of the membrane. Although small molecular weight substrates have been shown to diffuse through these membranes, such passage for starch and other polymers must be limited. Hydrolysis of starch probably occurs exclusively with the enzyme bound to the surface of either membrane.

Binding of amylase to the collagen membrane increases the resistance of the enzyme to denaturation and markedly amplifies the effect of temperature on the rate of reaction compared to the soluble enzyme. Increased resistance to thermal inactivation has been observed for a number of other enzymes immobilized on a variety of supports (Zaborsky, 1973).

In studies also using a collagen membrane, it was shown that the activation energy for immobilized lysozyme (Venkatasubramanian, 1972a, b) and lactose (Eskamani, 1972) increased by a factor of two. In the present study, a two-fold increase in activation energy was also observed. The effect of temperature on the rate of catalysis by several immobilized enzymes has been summarized by Vieth and Venkatasubramanian (1974). The temperature coefficient for the reaction apparently can remain unchanged or become greater or smaller upon immobilization. Melrose (1971) has attempted to explain these observations by suggesting

Table 2—Comparison of impregnation, codispersion and tanning on membrane activity

Membrane		Tanning with glutaraldehyde				Age (days)	Rate	Activity Remaining %
Casting pH	Technique	%	pH	Exposure, sec	Rate			
4.5	Imp	1	4.0	10	483	6	762	158
4.5	Imp	5	4.0	10	279	21	280	100
						43	169	61
11	Imp	10	8.0	15	360			
11	Imp	10	8.0	30	0			
11	Imp	10	8.0	120	0			
4.5	Cod	10	8.0	15	0			
4.5	Cod	10	8.0	30	0			
4.5	Cod	5	4.5	15	726	9	193	27
4.5	Cod	5	8.0	15	735	9	126	17
4.5	Cod	5	8.5	10	1095	9	120	11
4.5	Cod	1	8.5	30	904	9	170	19

the participation of influences on electron flow, local changes in polarity and introduction of strain.

Our studies indicate that some properties of the  $\alpha$ -amylase change when immobilized and that parameters such as pH, salt requirements and temperature must be optimized to obtain highest rates.

Although there is only a small change in the pH optimum, the yet unexplained requirement for chloride ions increases by 20-fold upon immobilization of  $\alpha$ -amylase on collagen. This change may be a manifestation of an altered amylase structure which requires a higher chloride concentration of the amylase upon immobilization or may reflect decreasing diffusional resistance due to increased swelling of the collagen as the chloride concentration increases. Alternately, since the activity of the soluble enzyme begins to fall off when the level of chloride exceeds 0.01M, and that of the immobilized enzyme only beyond 0.20M it may be that the collagen membrane exerts a protective effect by absorbing chloride ions and minimizing the actual local chloride concentration.

Of significance to long term use of the membrane was the finding that the immobilized enzyme was more resistant to thermal inactivation and exhibited a marked increase in the rate of hydrolysis

as the temperature was raised. It is apparent that these membranes are readily and inexpensively prepared, especially when compared to covalently derivatized immobilized enzymes. The membrane feature permits the development of reactor modules with flow-through properties which are particularly suited for continuous reaction and industrial scale processes.

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## REPLICATED COMPOSITE COMPLETE-INCOMPLETE BLOCK DESIGNS FOR SENSORY EXPERIMENTS

### INTRODUCTION

COMPOSITE complete-incomplete (C-I) block designs were introduced by Cornell and Knapp (1972) for use in sensory testing experiments where blocks refer to panelists or judges. Composite C-I block designs are formed by combining complete blocks of  $t$  units each, where  $t$  is the number of treatments to be compared, with balanced incomplete blocks of  $k$  units each ( $1 \leq k < t$ ) resulting in blocks of size  $t+k$  units. For example, two composite C-I block designs which might be considered with the three treatments A, B and C are as follows (for simplicity of illustration, only three blocks are listed); with  $k=1$ ,

Block	1	A	B	C	C
	2	A	B	C	A
	3	A	B	C	B

whereas with  $k=2$

Block	1	A	B	C	A	C
	2	A	B	C	B	C
	3	A	B	C	A	B

The principal advantage to using composite C-I blocks, say  $b$  of them, of size  $t+k$  units is that an estimate of pure experimental error from the  $bk$  duplicates can be obtained separately from a measure of the block-treatment interaction. It was shown by Cornell and Knapp, that by removing the interaction variation (however small) from the residual variation when comparing fixed treatment effects, the use of the experimental error only results in a more efficient test than can be attained with either the complete block or incomplete block designs. With these latter two designs, the error cannot be estimated separately from the interaction variation.

Cornell and Knapp (1972) discuss the application of composite C-I block designs in sensory experiments where a number of samples or treatments are compared subjectively by several panelists (blocks). It was assumed that the evaluation of the  $t$  samples by the  $b$  panelists (the number  $b$  could be large, say,  $b = 20, 30$ , or more) was performed at one sitting or in a single replication and thus only  $k$  of the samples were duplicated with each panelist. Often however, it may be advantageous to have every sample duplicated with every panelist to achieve a balance between panelists and samples, and therefore two more replications of the entire set of  $b(t+k)$  experiments is needed. To illustrate, consider the three panelists in the previous example with  $k=1$  where treatment C is duplicated with the first panelist, A with the second panelist and B with the third. Let us assume superficially that panelist 1 prefers treatment C to both treatments A and B. Owing to the duplication of treatment C with panelist 1, the average rating by panelist 1 is likely to be higher than if either treatment A or B had been duplicated. Hence the difference among the panelists is a combination of the partiality of treatment C by panelist 1 and the inherent difference among the panelists, each of which cannot be estimated separately.

Another minor drawback to these unreplicated C-I block designs arises when it is desired to compare treatments within each individual panelist for the purpose of interpreting panel-

ists  $\times$  treatments interaction. For example, in the previous example with  $k=1$ , suppose it is desired to compare the averages of treatments A and C within panelist 1. Since the average value for treatment C is based on twice as many observations as is the average value for treatment A, the average for treatment C will be twice as precise. Similarly, the average for A is twice as precise as the averages for B and C within panelist 2. Therefore in order that each treatment appears the same number of times with each panelist (as well as satisfy the requirement that each treatment be duplicated with every panelist at least once), then at least two additional replications would be needed. Three replications (denoted by I, II and III) of the C-I design in the previous example with  $k=1$  might be,

		I	II	III
Panelist	1	ABCC	ABCA	ABCB
	2	ABCA	ABCB	ABCC
	3	ABCB	ABCC	ABCA

For  $k=2$ , the replicated design might be,

		I	II	III
Panelist	1	ABCAC	ABCBC	ABCAB
	2	ABCBC	ABCAB	ABCAC
	3	ABCAB	ABCAC	ABCBC

In the incomplete block portion of the  $k=2$ , replicated C-I block design, we note that each and every pair of treatments (AB, AC and BC) occurs with each panelist over the three replicates. That every pair of treatments occurs together in one of the replicates with every panelist is a desirable property but arises as a result of using three replicates with three treatments when  $k=2$ . If  $t=4$  and  $k=2$ , then  $\binom{t}{k} = \binom{4}{2} = 6$  replicates would be needed to ensure that every pair of treatments appear together in one of the replicates with every panelist whereas only  $\frac{t}{k} = \frac{4}{2} = 2$  replicates would be required to duplicate the four treatments with each panelist. [The combinatorial symbol  $\binom{t}{k}$  denotes the number of combinations of  $t$  things taken  $k$  at a time and is computed as  $\frac{t(t-1)(t-2)\dots 1}{[k(k-1)\dots 1][(t-k)(t-k-1)\dots 1]}$ .]

Since one of our objectives in this paper is the comparison of treatments within each panelist and since we should like to keep the number of replications to a minimum owing to cost and time, we shall hereafter limit our discussion to the cases where if  $t$  is odd, then  $k=1$  and if  $t$  is even, then  $k=1, 2, \dots, \frac{t}{2}$ . By limiting ourselves to  $k=1$  when  $t$  is odd, the number of replications necessary for every panelist to duplicate every treatment will be exactly equal to  $t$  the number of treatments, whereas if  $k=1, 2, \dots, \frac{t}{2}$ , then the number of replications  $r$  must be an integer ( $r = \frac{t}{k}$ ). The number of panelists or blocks remains some multiple of  $\binom{t}{k}$ .

We now discuss the intrablock analysis of the replicated composite C-I block designs with particular emphasis on the estimation and testing of the panelists  $\times$  treatments interaction effects. Also, since we have introduced replications for the sole purpose of enabling each panelist the opportunity to duplicate every treatment, we shall assume that the replica-

tions do not contribute heavily to the total variation in the data. This assumption allows us the convenience of using a statistical model which is identical in form and notation to the model considered in the previous paper by Cornell and Knapp (1972). In the last section, we comment briefly on the use of a model with a replication term.

**INTRABLOCK ANALYSIS**

IN THE INTRABLOCK analysis, both the treatments and the panelists are considered fixed effects and the statistical model is written as

$$Y_{ij\ell m} = \mu + \tau_i + p_j + I_{ij} + \epsilon_{ij\ell m} \quad \begin{matrix} i=1,2,\dots,t \\ j=1,2,\dots,b \\ \ell=1,2,\dots,r = \frac{t}{k} \\ m=1 \text{ or } 2 \end{matrix} \quad (2.1)$$

where  $Y_{ij\ell m}$  is the  $m^{\text{th}}$  response to the  $i^{\text{th}}$  treatment by the  $j^{\text{th}}$  panelist in the  $\ell^{\text{th}}$  replication. The other terms in (2.1) are  $\mu$ , the overall mean;  $\tau_i$ , the effect of the  $i^{\text{th}}$  treatment;  $p_j$ , the effect owing to the  $j^{\text{th}}$  panelist;  $I_{ij}$ , the interaction parameter associated with the responses to the  $i^{\text{th}}$  treatment by the  $j^{\text{th}}$  panelist and  $\epsilon_{ij\ell m}$ , the random error associated with  $Y_{ij\ell m}$ . The errors  $\epsilon_{ij\ell m}$  are assumed to be randomly sampled from a Normal population with mean zero and variance  $\sigma_e^2$ .

The intrablock estimates of the effects  $\tau_i$ ,  $p_j$  and  $I_{ij}$  in model (2.1) are obtained using the following formulae (the hat “ $\hat{\cdot}$ ” denotes estimate),

$$\begin{aligned} \hat{\tau}_i &= \frac{k}{bt(k+t)} [tT_i - G] \\ \hat{p}_j &= \frac{k}{bt(k+t)} [bP_j - G] \\ \hat{I}_{ij} &= \frac{k}{(k+t)} [Y_{ij..} - \frac{T_i}{b} - \frac{P_j}{t} + \frac{G}{bt}] \end{aligned} \quad (2.2)$$

where  $T_i$  is the total of the responses to the  $i^{\text{th}}$  treatment,  $P_j$  is the total of the responses by the  $j^{\text{th}}$  panelist,  $Y_{ij..}$  is the total (summed over all replications) of the responses to the  $i^{\text{th}}$  treatment by the  $j^{\text{th}}$  panelist and,  $G$  is the grand total of all  $rb(k+t)$  observations. As a result of replicating the experiments, the estimates of the treatment effects are independent of the estimates of the panelists effects in (2.2).

Under the assumption the errors  $\epsilon_{ij\ell m}$  are uncorrelated with homogeneous variance  $\sigma_e^2$ , we have for the variance of  $\hat{\tau}_i$ , the covariance ( $\hat{\tau}_i, \hat{\tau}_{i'}$ ),  $i \neq i'$ , the variance of  $\hat{p}_j$  and covariance ( $\hat{p}_j, \hat{p}_{j'}$ ),  $j \neq j'$ ,

$$\text{Var}(\hat{\tau}_i) = \frac{k(t-1)}{bt(k+t)} \sigma_e^2, \text{Cov}(\hat{\tau}_i, \hat{\tau}_{i'}) = \frac{-1}{t-1} \text{Var}(\hat{\tau}_i), i \neq i' \quad (2.3)$$

$$\text{Var}(\hat{p}_j) = \frac{k(b-1)}{bt(k+t)} \sigma_e^2, \text{Cov}(\hat{p}_j, \hat{p}_{j'}) = \frac{-1}{b-1} \text{Var}(\hat{p}_j), j \neq j' \quad (2.4)$$

Before proceeding, let us remark on the variance  $\sigma_e^2$  above in (2.3) and (2.4). In the unreplicated composite C-I block designs, as with the replicated composite designs discussed in this paper, the experimental error variance  $\sigma_e^2$  represents the variation among the responses to the same treatment by the same panelist. In the unreplicated designs, an estimate of  $\sigma_e^2$  was obtained by first computing the range  $d_{ij}$  of the responses to the  $i^{\text{th}}$  treatment by the  $j^{\text{th}}$  panelist and then using the formula

$$\hat{\sigma}_e^2 = \frac{t}{b} \sum_{j=1}^b \frac{d_{ij}^2}{2bk} \quad (2.5)$$

The estimate  $\hat{\sigma}_e^2$  in (2.5) is based on  $bk$  degrees of freedom. With the replicated composite block designs on the other hand, each replicate of

the  $b$  panelists will provide an estimate similar to (2.5) and therefore over the  $r$  replications,

$$\hat{\sigma}_e^2 = \frac{r}{\ell=1} \sum_{i=1}^t \sum_{j=1}^b \frac{d_{ij}^2}{2bkr} \quad (2.6)$$

where  $d_{ij}$  is the range of the observations of the  $i^{\text{th}}$  treatment by the  $j^{\text{th}}$  panelist in the  $\ell^{\text{th}}$  replication. Furthermore, we shall assume the  $r$  replicates are consistent with respect to differences among the treatments, differences among the panelists, as well as the source panelists  $\times$  treatments interaction and therefore the intrablock analysis of variance table will take the form shown in Table 1. By “consistency of the replications” is meant the treatment differences, panelist differences and the panelists  $\times$  treatments interaction effects remain relatively constant from replication to replication and therefore do not interact with replications. The source of variation in Table 1 termed residual is a pooled measure of replication variation and replication interaction variation and although we have assumed these measures of variation to be negligible, we shall calculate the sum of squares residual separate from the sum of squares error in (2.6) in order to test our assumption. The sum of squares residual is computed using

$$\begin{aligned} \text{Sum of squares residual} &= \sum_{i=1}^t \sum_{j=1}^b \sum_{\ell=1}^r \sum_m Y_{ij\ell m}^2 - t \sum_{i=1}^t \sum_{j=1}^b \frac{Y_{ij..}^2}{r(k+t)} \\ &\quad - \text{Sum of squares error.} \end{aligned} \quad (2.7)$$

Sum of squares error equals  $bkr$  times  $\hat{\sigma}_e^2$  in (2.6).

To test our assumption of the nonexistence of both replication variation as well as replication interaction variation, a preliminary  $F$ -test of significance (usually at the .05 level) is performed using the ratio

$$F = \frac{\text{Mean square residual}}{\text{Mean square error}} \quad (2.8)$$

and the value in (2.8) is compared to the corresponding value of  $F$  (with  $bt(r-1)$  and  $rbk$  degrees of freedom respectively) from the tables. With a nonsignificant ratio (2.8), the residual sum of squares and the error sum of squares are combined (or pooled) to form a new estimate of  $\sigma_e^2$ , this latter estimate being based on  $rb(k+t) - bt$  degrees of freedom,

$$\text{New mean square error} = \frac{\text{S.S. residual} + \text{S.S. error}}{rb(k+t) - bt} \quad (2.9)$$

Having performed the preliminary test (2.8) and formed the pooled estimate of  $\sigma_e^2$  in (2.9), we now test the significance of the treatment effects, the panelists effects, as well as the panelists  $\times$  treatments interaction effects. These tests are each performed, again with an  $F$  ratio, using the respective mean squares in Table 1 against the new mean square error in (2.9). The test on the magnitude of the interaction variation is performed first, since a significant interaction among panelists with respect to treatment differences implies the panelists are not in agreement in their evaluations of the treatment differences. If the test for interaction is not significant, tests on treatment differences and panelists differences are then performed.

If the test for interaction variation is significant, we must look further into the interaction effects which attribute to the significance of the  $F$  test. Since panelists  $\times$  treatments interaction is not uncommon, particularly when untrained panelists are used in the treatment evaluations, we shall now discuss a method which can be used to analyze interaction effects. Following that, we shall illustrate the methodology using a numerical example.

**ANALYZING PANELISTS  $\times$  TREATMENTS INTERACTION**

IN TWO-FACTOR experiments (such as panelists by treatments), interaction effects can be interpreted more easily by concentrating on the interaction elements which make up interaction effects. Let us denote the average response to the  $i^{\text{th}}$  treatment by the  $j^{\text{th}}$  panelist by  $\bar{\tau}_{ij}$ .

Table 1—Intrablock analysis of variance

Source	d.f.	S.S.	Expected M.S.
Treatments	$t-1$	$\frac{t}{t} \sum_{i=1}^t \frac{T_i^2}{rb(k+t)} - \frac{(\sum_{i=1}^t T_i)^2}{rb(k+t)}$	$\sigma_e^2 + \frac{b(k+t)}{k(t-1)} \sum_i \tau_i^2$
Panelists	$b-1$	$\frac{b}{j=1} \sum \frac{P_j^2}{r(k+t)} - \frac{(\sum_{j=1}^b P_j)^2}{rb(k+t)}$	$\sigma_e^2 + \frac{t(k+t)}{k(b-1)} \sum_j p_j^2$
PxT Interaction	$(b-1)(t-1)$	$\frac{t}{i=1} \sum \frac{b}{j=1} \sum \frac{Y_{ij}^2}{r(k+t)} - \frac{\sum_{j=1}^b P_j^2}{r(k+t)}$ — S.S. treatments	$\sigma_e^2 + \frac{(k+t)}{(b-1)(t-1)} \sum_{ij} I_{ij}^2$
Residual	$bt(r-1)$	(2.7)	
Error	$rbk$	(2.6) X bkr	$\sigma_e^2$

Then interaction elements are of two types; those of the form  $I_{ij} - I'_{ij} = \bar{\tau}_i p_j - \bar{\tau}'_i p_j$ , representing the difference between the averages of two treatments (i and i') with the same panelist (the j<sup>th</sup>) and called type 1 say, and those of the form  $I_{ij} - I'_{ij} = \bar{\tau}_i p_j - \bar{\tau}'_i p'_j$ , representing the difference between the average responses of two panelists (j and j') with the i<sup>th</sup> treatment and called type 2. Now an interaction between treatments i and i' with panelists j and j' can be expressed as the difference of two interaction elements of type 1 or as the difference between two interaction elements of type 2 as follows,

$$\begin{aligned} (I_{ij} - I'_{ij}) - (I_{ij'} - I'_{ij'}) &= (\bar{\tau}_i p_j - \bar{\tau}'_i p_j) - (\bar{\tau}_i p_{j'} - \bar{\tau}'_i p_{j'}) \\ &= (\bar{\tau}_i p_j - \bar{\tau}'_i p_{j'}) - (\bar{\tau}'_i p_j - \bar{\tau}'_i p_{j'}) \\ &= (I_{ij} - I'_{ij'}) - (I'_{ij} - I'_{ij'}) \end{aligned} \quad (3.1)$$

In a  $t \times b$  experiment, there are  $\binom{t}{2} \binom{b}{2}$  such individual interactions to be considered. For fixed i and i', there are  $\binom{b}{2}$  groups where each group consists of b interaction elements of type 1,

$$I_{ij} - I'_{ij'}, \text{ for } j=1, 2, \dots, b, \quad (3.2)$$

and for fixed j and j', there are  $\binom{t}{2}$  groups each consisting of t interaction elements of type 2,

$$I_{ij} - I'_{ij'} \text{ for } i=1, 2, \dots, t. \quad (3.3)$$

The difference between any two interaction elements in the same group is an interaction, but the difference between two interaction elements of different types,

$$(I_{ij} - I'_{ij'}) - (I_{ij} - I'_{ij'}) = I_{ij'} - I'_{ij}$$

or of the same type but in different groups,

$$(I_{ij} - I'_{ij'}) - (I_{ij'} - I'_{ij'}) = I_{ij} - I'_{ij} - I_{ij'} + I'_{ij'}$$

is of no interest. Hence we are not interested in testing all  $\binom{t}{2} \binom{b}{2}$  individual interactions simultaneously but rather we shall concentrate on performing  $\binom{b}{2}$  tests where each test is made on the  $\binom{b}{2}$  differences chosen among the b interaction elements of type 1.

To illustrate, let us consider three treatments A, B and C and again

for simplicity purposes, only three panelists 1, 2 and 3. In the two-way table of means below, the numbered entries represent the averages of  $\binom{k+t}{k}$  observations which are estimates of the  $I_{ij}$ . Interaction elements of type 1 are  $I_{Aj} - I_{Bj}$  for  $j=1, 2$  and  $3$ , that is,  $I_{A1} - I_{B1} = 7.5 - 6.0 = 1.5$ ,  $I_{A2} - I_{B2} = 5.0 - 8.0 = -3.0$  and  $I_{A3} - I_{B3} = 2.5$ ; and similarly for  $I_{Aj} - I_{Cj}$  and  $I_{Bj} - I_{Cj}$ ,  $j=1, 2$  and  $3$ .

Treatment	A	B	C
Panelist 1	7.5	6.0	4.0
2	5.0	8.0	7.0
3	6.0	3.5	7.0

Since  $t=3$ , we shall need to perform  $\binom{3}{2}=3$  tests where each test is performed on  $\binom{b}{2}=\binom{3}{2}=3$  differences where each difference is between a pair of panelists means.

For test 1, the interaction elements to be compared are computed from the difference  $\bar{\tau}_A - \bar{\tau}_B$  for each panelist, that is,

$$(\bar{\tau}_A - \bar{\tau}_B) = \begin{matrix} \bar{p}_1 & \bar{p}_2 & \bar{p}_3 \\ 1.5 & -3.0 & 2.5 \end{matrix} \quad (3.4)$$

For test 2, the difference  $\bar{\tau}_A - \bar{\tau}_C$  is similarly computed for each panelist

$$(\bar{\tau}_A - \bar{\tau}_C) = \begin{matrix} \bar{p}_1 & \bar{p}_2 & \bar{p}_3 \\ 3.5 & -2.0 & -1.0 \end{matrix} \quad (3.5)$$

and for the third test,

$$(\bar{\tau}_B - \bar{\tau}_C) = \begin{matrix} \bar{p}_1 & \bar{p}_2 & \bar{p}_3 \\ 2.0 & 1.0 & -3.5 \end{matrix} \quad (3.6)$$

Now the interactions formed by taking the differences  $\bar{p}_1 - \bar{p}_2$ ,  $\bar{p}_1 - \bar{p}_3$  and  $\bar{p}_2 - \bar{p}_3$  in (3.4) [as well as in (3.5) and (3.6)] are each a difference of two interaction elements and hence each interaction is an algebraic sum of the means of four samples where each sample is of size

$k+t/k$ . Thus if the standard error of each interaction element is given by  $\sigma_{IE} = (2k\sigma^2/k+t)^{1/2}$ , where  $\sigma^2$  is estimated using the new Error Mean Square, then the standard error of each interaction is  $\sqrt{2} \sigma_{IE}$ .

To determine which interaction effects are large, a multiple comparison procedure is used on the interaction elements in (3.4). Some of the more popular multiple comparison procedures in use today are Duncan's new multiple range test, the Newman-Keuls procedure, Tukey's Honestly Significant Difference procedure and the Least Significant Difference (multiple t) test; see Cornell (1972) for a review of some multiple comparison procedures. The multiple comparison procedure is used on the interaction elements in (3.5) and (3.6) as well and the results of the three tests are then combined in an attempt to understand more completely the significance of the panelists  $\times$  treatments interaction.

We now illustrate the intrablock analysis with a numerical example. We shall use Duncan's new multiple range test as an aid to interpreting the interaction effects. To keep the example short so that we may present all the necessary calculations, we again use only three panelists and three treatments.

**AN EXAMPLE OF THE INTRABLOCK ANALYSIS**

THREE PANELISTS 1, 2 and 3 were asked to evaluate three strawberry preserves (treatments A, B and C) using numerical values from 1 to 9 according to the amount of fresh strawberry flavor in each. Three replicates (I, II and III) of the experiment were performed and only a single treatment was duplicated with each panelist in each replicate. The data and the corresponding two-way table of panelists and treatment totals are,

Treatment	Rep I			Rep II			Rep III		
	A	B	C	A	B	C	A	B	C
Panelist 1	5,6	6	3	7	5,4	2	6	4	3,1
2	4	7,7	3	5	7	2,4	4,2	6	4
3	6	4	4,6	8,6	5	3	7	3,3	4

Treatment	A	B	C	$P_j$	$\hat{p}_j$
Panelist 1	24	19	9	52	-0.28
2	15	27	13	55	-0.03
3	27	15	17	59	0.31
$T_i$	66	61	39	166	
$\hat{\tau}_i$	0.89	0.47	-1.36		

The estimates of the effects of treatment A and panelist 1 are, from (2.2),

$$\hat{\tau}_A = 66/12 - 166/36 = 0.89, \hat{p}_1 = 52/12 - 166/36 = -0.28. \quad (4.1)$$

From Table 1, the sum of squares treatments, sum of squares panelists and sum of squares  $P \times T$  interaction respectively are,

$$\text{S.S. treatments} = 1/12 [(66)^2 + (61)^2 + (39)^2] - (166^2/36) = 34.39$$

$$\text{S.S. panelists} = 1/12 [(52)^2 + (55)^2 + (59)^2] - (166^2/36) = 2.06$$

$$\text{S.S. } P \times T \text{ interaction} = 1/4 [(24)^2 + (15)^2 + \dots + (17)^2] - 1/12 [(52)^2 + (55)^2 + (59)^2] - 34.39 = 44.09.$$

S.S. error and S.S. residual are computed using (2.6) and (2.7) respectively,

$$\text{S.S. error} = 1/2 [(5 - 6)^2 + (7 - 7)^2 + \dots + (3 - 1)^2] = 11.00,$$

$$\text{S.S. residual} = 5^2 + 6^2 + \dots + 4^2 - 1/4 [(24)^2 + (15)^2 + \dots + (17)^2] - 11.00 = 15.00. \quad (4.2)$$

and the preliminary F-test on the residual mean square is,

$$F = \frac{15.00/13}{11.00/9} = 0.68 \quad (4.3)$$

which is clearly not significant and therefore the new pooled estimate of  $\sigma_e^2$  using (2.9) is,

$$\hat{\sigma}_e^2 = \frac{15.00 + 11.00}{18 + 9} = 0.963. \quad (4.4)$$

The test on the magnitude of the panelists  $\times$  treatments interaction effects (using (4.4) in the denominator of the F ratio) is  $F = (44.09/4)/0.963 = 11.4$  which is significant at the 0.01 level and therefore we shall investigate further the magnitudes of the individual interaction effects. Using the previous two-way table of totals, we construct the following two-way table of means where each mean is computed using  $(k+t)/k = 4$  observations,

Treatments	A	B	C
Panelists 1	6.00	4.75	2.25
2	3.75	6.75	3.25
3	6.75	3.75	4.25

and also the following two-way table of the estimates of the interaction elements of type 1,

Treatment difference	A-B	A-C	B-C
Panelist 1	1.25	3.75	2.50
2	-3.00	0.50	3.50
3	3.00	2.50	-0.50

To use Duncan's new multiple range test, we arrange the interaction elements in each column of the latter table by putting them in descending order of magnitude. The estimated standard error of each entry in the table is  $\hat{\sigma}_{IE} = (0.963)^{1/2} = 0.695$ , and from a table of Duncan values (Harter, 1960; Steel and Torrie, 1960), the significant ranges at the 0.05 level, for tests of ordered means 2 and 3 steps apart with 27 degrees of freedom for the standard error of the mean, are respectively 2.90 and 3.05. Hence, the range of all three interaction elements in a column of the latter table will be judged significant at the 0.05 level if it exceeds  $(3.05)(0.695) = 2.12$ , while the difference of two adjacent ordered interaction elements will be judged significant if it exceeds  $(2.90)(0.695) = 2.02$ . The results of the three tests are presented below where if two or more interaction elements are underscored by the same line, they are not significantly different (at the 0.05 level of significance).

Test 1: A-B	<u>3.00</u>	<u>1.25</u>	-3.00	<u>3</u>	<u>1</u>	2
Test 2: A-C	<u>3.75</u>	<u>2.50</u>	0.50	<u>1</u>	<u>3</u>	2
Test 3: B-C	3.50	<u>2.50</u>	-0.50	<u>2</u>	<u>1</u>	3

In tests 1 and 2, we see that panelists 1 and 3 are similar (not different) in their evaluation of the difference between A and B, but panelist 2 is different from 1 and 3 as he rates B higher than A, but C and A about equal. In test 3, panelists 1 and 2 are similar in rating B higher than C, but panelist 3 rates B and C about equal. Thus panelists 1 and 3 agree that the flavor of treatment A is rated higher than the flavors of both B and C while panelist 2 does not agree and in fact scores B higher than A. Here an interaction is present between the preserves A and B with panelists 1 and 2 as well as with panelists 2 and 3. Also, panelists 1 and 2 score B higher than C while panelist 3 is undecided about B and C.



Confronted with this lack of consistency or agreement among the panelists in their scoring of the treatments, we might repeat the three replicates of the  $b(k+t)$  experiments with the same panelists and check the validity of the interaction or we might select new panelists and combine the data from the two sets of experiments. In this latter case, if five panelists (1 and 3 plus the three new panelists, say) score A higher than B and C, perhaps this evidence will reduce, to a degree, the significance of the interaction arising as a result of the scoring of B higher than A by panelist 2.

We now discuss briefly the analysis of the mixed model where the treatments remain fixed but the blocks (panelists) are considered to be a random sample taken from a large population of blocks. As an example, 60 students were asked to rate five commercial peanut butters according to flavor. It was assumed this large number of students would adequately reflect the opinions of the general public and it was of interest to estimate the variation among their flavor ratings.

MIXED MODEL ANALYSIS

WHEN THE PANELISTS used in the experiment are assumed to be a representative random sample from a large population of panelists, the emphasis is on estimating the variances  $\sigma_p^2$  and  $\sigma_i^2$  of the populations of panelists and interaction random variables from which the samples were taken. The analysis of variance table for the mixed model is identical in form to Table 1 with the exception of the expected mean square formulas. The formulas for the expectations of the mean squares with the mixed model are,

$$\text{E.M.S. treatments} = \sigma_a^2 + \frac{(k+t)}{k} \sigma_i^2 + \frac{b(k+t)}{k(t-1)} \sum_{i=1}^t \tau_i^2, \quad (5.1)$$

$$\text{E.M.S. panelists} = \sigma_e^2 + \frac{(k+t)}{k} \sigma_i^2 + \frac{t(k+t)}{k} \sigma_p^2 \quad (5.2)$$

$$\text{E.M.S. P} \times \text{T interaction} = \sigma_e^2 + \frac{(k+t)}{k} \sigma_i^2 \quad (5.3)$$

$$\text{E.M.S. new error} = \sigma_e^2. \quad (5.4)$$

To test the hypothesis,  $P \times T$  interaction variation is zero (that is,  $\sigma_i^2 = 0$ ), the ratio  $F = (\text{M.S. } P \times T \text{ interaction})/(\text{M.S. new error})$  is compared against a tabular  $F$  value with  $(b-1)(t-1)$  and  $rb(k+t)-bt$  degrees of freedom respectively, assuming the preliminary test (2.8) is not significant. A significant test for interaction would imply that the variation among the panelists used in the experiment when comparing treatments  $i$  and  $i'$  is different from the variation among the panelists when comparing treatments  $i$  and  $i''$ . Stated another way, the variation among the  $b$  panelists is not constant with each of the  $\binom{t}{2}$  tests involving the interaction elements of type 1.

To estimate the variance components  $\sigma_p^2$  and  $\sigma_i^2$  we have from (5.2), (5.3) and (5.4),

$$\hat{\sigma}_i^2 = k/k+t [\text{Mean square interaction} - \text{Mean square new error}], \quad (5.5)$$

$$\hat{\sigma}_p^2 = k/t(k+t) [\text{Mean square panelists} - \text{Mean square interaction}]. \quad (5.6)$$

The formulae (5.5) and (5.6) are considerably easier to use than are the corresponding formulae with the unreplicated composite C-I block designs. Furthermore, to test the hypothesis that the differences among the treatment effects are small relative to the magnitude of the interaction variation, an exact  $F$  test can be performed using the ratio,

$$F = \frac{\text{Mean square treatments}}{\text{Mean square interaction}} \quad (5.7)$$

and this  $F$  value is compared to the tabular  $F$  value with  $t-1$  and  $(b-1)(t-1)$  degrees of freedom respectively. That the test (5.7) is an exact test is another reason for replicating these designs since with the unreplicated composite block designs, the  $F$  test corresponding to (5.7) was an approximate test.

CONCLUDING REMARKS

IN THIS PAPER we have discussed the analysis of composite complete-incomplete block designs where the  $b$  blocks (panelists) are replicated. The main reason for replicating the  $b(k+t)$  experiments was to enable each panelist the opportunity to duplicate each of the treatments at least once as well as satisfy the condition that every treatment appear the same number of times with each panelist. This latter condition is essential in order to accurately interpret panelists  $\times$  treatments interaction effects.

Throughout the paper we have seen that with the replicated composite C-I block design, the formulae for calculating the estimates of the treatment effects, the panelists effects, as well as the panelists component of variance, are simpler in form than the corresponding formulae with the unreplicated composite block designs. Perhaps not so obvious was the simpler form in (2.2) of the formulae for calculating estimates of the interaction effects as well as the simple form in Table 1 for calculating S.S.  $P \times T$  interaction. These simpler forms arose as a result of our assuming negligible replication variation and therefore of not including a term for replication effects in the model (2.1).

If the assumption of negligible replication variation is not valid, a replication term, say  $r\varrho$ , is included in the model (2.1). With the additional restriction that the replication effects sum to zero, the estimate of the effect of the  $l^{\text{th}}$  replicate as well as the estimate of  $I_{ij}$  would be of the form,

$$\hat{r}_l = \frac{r(k+t)}{br(k+t)^2 - kt} [R_l - \frac{tG}{r(k+t)} - \frac{t}{r(k+t)} \sum_{i=1}^t \sum_{j=1}^b \phi_{ijl} Y_{ij..}]$$

$$\hat{I}_{ij} = \frac{t}{r(k+t)} [Y_{ij..} - \frac{T_i}{b} - \frac{P_j}{t} + \frac{G}{bt} - \sum_{i=1}^t \sum_{j=1}^b \phi_{ijl} \hat{r}_l],$$

where  $\phi_{ijl} = 0$  or 1 depending on the absence or presence of the duplicate of the  $i^{\text{th}}$  treatment with  $j^{\text{th}}$  panelist in the  $l^{\text{th}}$  replicate. The estimates  $\hat{r}_l$  and  $\hat{I}_{ij}$  are not independent and therefore the formulae for S.S. replications and S.S.  $P \times T$  interaction are also not independent. Consequently, S.S. replications would have to be adjusted for the interaction effects and similarly, S.S.  $P \times T$  interaction would have to be adjusted for replication effects.

Finally, if there is evidence to indicate that replicate effects are different when the replication mean square is compared against the error mean square, it becomes extremely difficult to determine what effect the replicate effects have on the panelists  $\times$  treatments interaction effects. This is because some replicate effects which are present in certain type 1 interaction elements are not present in other type 1 interaction elements, and when comparing interaction elements to estimate interaction effects, the different replicate effects enter into the comparisons. Hence our reason for assuming negligible replication variation throughout the paper. It should not be too much to ask for consistent repetition of experimental conditions.

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## REFLECTANCE COLORIMETRIC EVALUATION OF EGG YOLK PIGMENTATION

### INTRODUCTION

NUMEROUS scoring systems have been developed for visual evaluation of egg yolk color or pigmentation. The Roche Yolk Colour Fan (Vuilleumier, 1969) is the most recently developed and most frequently used. Visual systems are not objective, the hue of the chart or fan and the sample may not match, and reproducibility from experiment to experiment and laboratory to laboratory is not good.

The egg products industry is routinely using the AOAC (1970) method of determining yolk color intensity; data from this procedure are expressed as mcg beta carotene per g of sample and are usually referred to as beta carotene equivalents. Other extraction procedures have included the NEPA number system (IAPI, 1968) and the ANRC method (Marusich, 1967). These extraction procedures have the disadvantage that (1) they are time consuming; (2) there is a high glassware requirement; (3) the data thus obtained give no indication of the hue of the sample; and (4) the "standard" and the sample may have different hues or dominant wavelengths.

Hartfield and Schmitt (1965) recommended that yolk color be evaluated on the basis of the CIE system. This study was undertaken to evaluate a reflectance colorimeter, the IDL (Instrument Development Laboratories) COLOR-EYE<sup>®</sup> (Signature Model), as a rapid and objective instrument for use in pigment determination of egg yolks.

### EXPERIMENTAL

#### Experiment 1

Eggs were obtained from two groups of hens, one group fed a commercial-type layer diet containing 26.4 mg/kg carotenoids supplied by yellow corn and alfalfa, and the other fed a similar but pigment-free diet. Eggs were broken and the contents were hand separated. After most of the albumen had been removed, the yolk was rolled across a dampened paper towel to remove the chalazae and adhering albumen.

24 samples exhibiting a wide range of pigmentation levels were prepared by various combinations of the "white" and pigmented yolks; the combinations ranged from 15:1 (white:pigmented) to 1:6 (white:pigmented). The samples, each totaling 30g, were mixed by hand with a spatula rather than a mixer to avoid incorporation of air bubbles. A portion of each sample was placed in lucite sample holders

identical in design to those described by Little (1969) but of smaller dimension, the well being 1.0 cm deep and 2.8 cm in diameter. Reflectance readings for X, X', Y and Z were determined on the COLOR-EYE based on the illuminant C and utilizing the white Vitrolite standard supplied with the instrument. From these readings luminous reflectance, excitation purity and dominant wavelength were calculated using the IBM 360/65 computer and a program prepared by Fry and Damron (1971).

Each sample was also extracted according to AOAC (1970) procedure. Absorption readings were compared to a beta carotene standard, thereby equating xanthophyll content to beta carotene equivalents. The relationships of beta carotene equivalents with excitation purity and luminous reflectance were plotted and statistically analyzed for regression coefficients.

#### Experiment 2

Yolks with varying xanthophyll content were evaluated with the COLOR-EYE (as in Experiment 1) before and after mixing 1:1 with a white diluent; this was done to investigate the possible beneficial effect of the dilution on providing a greater range in values for luminous reflectance and reducing the tendency of excitation purity to plateau at high levels of yolk intensity.

The yolks used were from hens receiving different levels and sources of dietary xanthophylls. Levels of 5 and 20 mg of xanthophyll per kg of feed were selected using alfalfa, yellow corn and corn gluten meal as sources. These ingredients, as listed, have increasing utilization

values, thus giving a spread in yolk pigmentation level.

As a result of several preliminary tests the diluent selected was an emulsion of 25% mineral oil with milk of magnesia. It was considered desirable to have a white rather than clear diluent; it had to mix readily with egg yolk, stay in suspension for an adequate length of time for measurement with some safety margin, and be as nearly white as possible so it would not impart its own color characteristics to the yolk sample. COLOR-EYE evaluation of the diluent used resulted in an excitation purity value of 0.62%, indicating that it was very nearly white. Excitation purity values for three samples each of three different lots of this product were as follows: #3C29, 0.40%, 0.38%, 0.52%; #2L27, 0.36%, 0.49%, 0.42%; #3E15, 0.75%, 0.77%, 0.81%.

### RESULTS & DISCUSSION

#### Experiment 1

Luminous reflectance ( $Y_{CIE}$ ) exhibited an inverse relationship with beta carotene equivalents with a resultant correlation coefficient of  $-0.874$  for the two parameters. The relationship was proven linear and the corresponding line of regression was drawn (Snedecor, 1956). The relationships of luminous reflectance values and beta carotene equivalents were then determined as presented in Table 1.

Table 1—Relationship of beta carotene equivalents (BCE) to excitation purity ( $P_e$ ) and luminosity ( $Y_{CIE}$ )

BCE <sup>a</sup>	$Y_{CIE}$	$P_e$ (%)	BCE	$Y_{CIE}$	$P_e$ (%)	BCE	$Y_{CIE}$	$P_e$ (%)
2.0	39.51	58.50	10.0	36.68	80.15	18.0	33.86	88.25
2.5		60.00	10.5		81.00	18.5		88.55
3.0	39.16	61.50	11.0	36.33	81.75	19.0	33.50	88.85
3.5		63.00	11.5		82.45	19.5		89.15
4.0	38.80	64.50	12.0	35.98	83.00	20.0	33.15	89.45
4.5		66.00	12.5		83.45	20.5		89.60
5.0	38.45	67.50	13.0	35.62	83.90	21.0	32.80	89.75
5.5		69.00	13.5		84.35	21.5		89.90
6.0	38.10	70.45	14.0	35.27	84.80	22.0	32.44	90.05
6.5		71.75	14.5		85.25	22.5		90.20
7.0	37.74	73.30	15.0	34.92	85.70	23.0	32.09	90.35
7.5		74.80	15.5		86.15	23.5		90.50
8.0	37.39	76.15	16.0	34.56	86.60	24.0	31.74	90.65
8.5		77.25	16.5		87.05	24.5		90.80
9.0	37.04	78.25	17.0	34.21	87.50	25.0	31.38	90.95
9.5		79.20	17.5		87.95			

<sup>a</sup> Beta carotene equivalents are in terms of mcg beta carotene per gram of sample (AOAC, 1970).

The slightly pigmented yolk samples had a luminous reflectance value of 39.51, whereas the other extreme in pigmentation resulted in a value of 31.03. Although the relationship of luminous reflectance to beta carotene equivalents was linear, a decided advantage for ease of comparison, the major disadvantage in the use of luminous reflectance for yolk pigmentation evaluation is the relatively small range or spread in the values.

Similar data for the relationship of beta carotene equivalents and excitation purity are also shown in Table 1. The relationship between these parameters was curvilinear but this function could be reduced to three linear components, the longest of which was at the lower levels of intensity. Corresponding regression lines were calculated, plotted and used to develop the data presented. The correlation coefficient for these two parameters was +0.906.

It can be noted that the range in excitation purity values for slightly pigmented to well pigmented yolk samples was from less than 60% to over 90%. This was a considerably greater range than noted for luminous reflectance and might make comparisons with beta carotene equivalents easier and more accurate. If excitation purity is to be used for such comparisons with beta carotene equivalents the most favorable range of measurement appears to be between 40% and 90%. A series of dilutions falling within this range would give consistent estimations for xanthophyll content. A definite

tendency for excitation purity values to plateau exists at beta carotene equivalents of 20 or higher. Experiment 2 was conducted to evaluate the use of a white diluent as a possible means of reducing this effect.

Experiment 2

Luminous reflectance values, dominant wavelength and excitation purity values were plotted for the diluted and nondiluted yolks (Fig. 1, 2 and 3). Lines were drawn to connect the points in order to determine whether or not parallel relationships existed for the samples of diluted and nondiluted yolks differing in pigmentation level.

The use of white diluent (1:1 ratio) increased the average luminous reflectance value from approximately 35 to approximately 61. With one exception (yellow corn at 5 mg/kg), the lines were very nearly parallel indicating that dilution increased luminous reflectance values in the same proportion for each sample. The range from high to low values was slightly less, however, for the diluted yolks than for nondiluted yolks. The problem of a limited range discussed for Experiment 1 was not solved by dilution.

Dominant wavelength was changed considerably for the higher levels of pigmentation but none for the lowest level. It had been expected that the use of a white diluent would have little or no effect on the dominant wavelength of the sample, with the primary effects being on excitation purity and luminous reflec-

tance. If reflectance colorimetric measurements of yolks were used commercially it had been thought that an egg products buyer might be able to specify delivery of product of a specific hue and intensity. If measurements are made on diluted yolks, adjustments would need to be made in the dominant wavelength specifications.

The yolk dilution reduced the tendency for the excitation purity to plateau. The lines for the four lower levels of xanthophyll content were relatively parallel but a substantial increase resulted for the two higher levels. The use of a white diluent did move all of the values downward to what is considered a more acceptable, and perhaps more accurate, range for excitation purity measurement. In a separate study where xanthophyll utilization was determined based on a standard of dietary beta-apo-8'-carotenal, almost identical utilization values were obtained with data from diluted and nondiluted yolks (Fry and Harms, 1973).

It is suggested that this procedure may have commercial application in egg product pigmentation evaluation. A reflectance colorimetric evaluation of egg products would have the potential of reducing time and glassware requirements and permit a buyer to specify hue as well as intensity.

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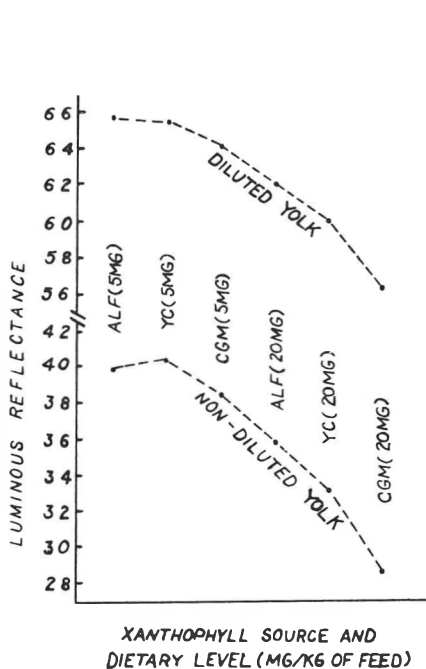


Fig. 1—Effect of a white diluent on yolk luminous reflectance.

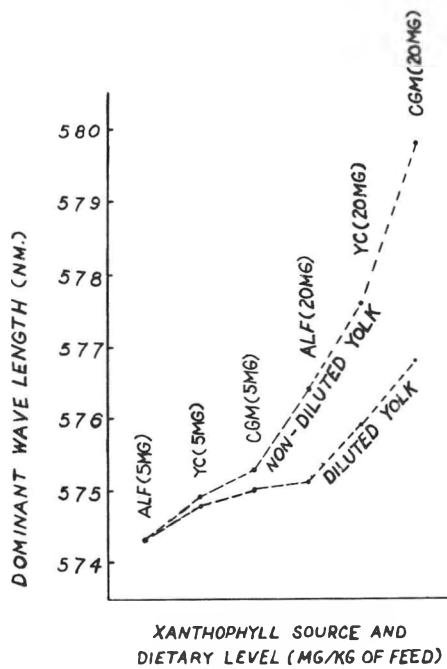


Fig. 2—Effect of a white diluent on yolk dominant wavelength.

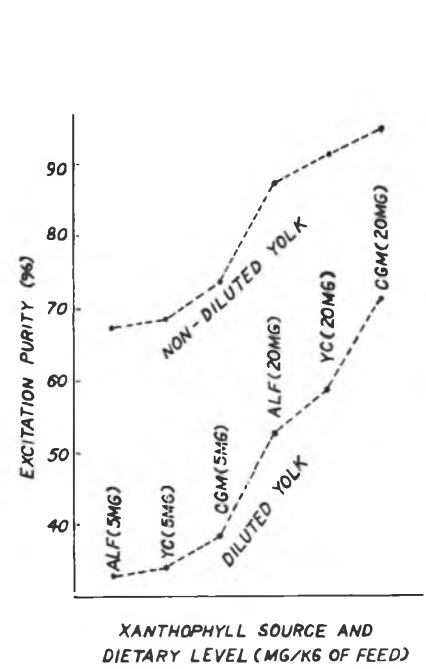


Fig. 3—Effect of a white diluent on yolk excitation purity.

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## CATHEPTIC ENZYME ACTIVITY IN AGED COUNTRY-STYLE HAMS AS INFLUENCED BY PRE-CURING TREATMENT

### INTRODUCTION

IMPROVED METHODS of processing hams have significantly increased the quantity and quality of commercially produced dry-cured hams. The effect has been to increase the income in recent years of many people in southeastern United States.

Demand for high quality country-style hams has stimulated research on this product. The research has been directed towards further improvement of commercial processing procedures in an effort to produce and maintain the distinctive flavor and aroma characteristics but in less time and with more consistent quality. Studies conducted on the flavor and aroma characteristics of dry-cured hams indicate that these changes are brought about by a series of chemical and biochemical reactions taking place during curing, smoking and storage (aging) periods (Howe and Barbella, 1937; Hunt et al., 1939; Callow, 1947; Jensen, 1949; Blumer, 1954; Tilgner, 1957; Kemp et al., 1961; Craig et al., 1964; Lawrie, 1966). These chemical reactions are affected by environmental factors such as temperature and relative humidity (Howe and Barbella, 1937; Hunt et al., 1939; Cecil and Woodroof, 1954; Blumer, 1958; Fields et al., 1955).

Catheptic enzymes are known to be responsible for autolysis in beef and pork muscle tissues as well as the increase in free amino acid concentration and non-protein nitrogen in irradiated bovine and porcine muscles (Drake et al., 1957; Zender et al., 1958; West et al., 1961; Sharp, 1963). Proteolytic enzymes probably contribute to the characteristic aged flavor of country-cured hams. Evidence indicates that changes in free amino acid concentration of aged country-style hams enhances flavor (McCain et al., 1968; Ambanelli and Fratti, 1970).

The present study was conducted to determine if there is catheptic activity in hams during curing, to determine the influence of aging time on catheptic activity and to study the relationship of amino acid production and catheptic activity among fresh, frozen, cured and aged products.

### EXPERIMENTAL

12 SHORT-CUT, skinned hams (6.3–7.7 kg) from hogs of unknown origin were purchased from a local meat packer for use in this study. These hams were assigned randomly to each of four lots which were then subjected to different storage conditions prior to curing. The treatments were fresh chilled or fresh chilled and cured immediately or following thawing after frozen storage at  $-29^{\circ}\text{C}$  for 2 days, 2 months and 1–3 yr. A randomized complete block design was used with the combination of sampling periods and pre-curing treatments. At each designated sampling period, a fresh ham was purchased from the same source and subjected to the same analyses for comparison to the cured hams.

#### Curing, aging and sampling

Hams were dry cured according to the method described by McCain et al. (1968). Hams were cured with 31g per 0.45 kg of ham of a dry curing mixture consisting of 3.63 kg sodium chloride, 0.91 kg white sucrose, 71g potassium nitrate and 14g of sodium nitrite. One-third of the curing mixture required for each ham was applied on the first, third and tenth days and the hams allowed to remain in cure at  $4^{\circ}\text{C}$  for 2 days/0.45 kg.

After curing the hams were washed free of surface curing mixture, dried approximately 24 hr at  $32^{\circ}\text{C}$ , placed in stockinettes and hung in a cooler at  $4^{\circ}\text{C}$  for 30 days to effect salt equalization. They were then aged at  $27^{\circ}\text{C} \pm 2$ , relative humidity,  $55\% \pm 4$  and air flow, 35 ft/min until sampled.

A ham was taken randomly from each lot and a 1 in. cross sectional slice removed approximately 1 in. ventral and parallel to the ischium (itch bone). The semimembranosus muscle was then dissected and trimmed free of the outer dried muscle area and subcutaneous fat. Thereafter, the muscle was ground through a  $\frac{1}{4}$  in. plate, packaged and stored at  $-29^{\circ}\text{C}$  until used for the determination of moisture, fat, nitrogen, salt, pH, free amino acids and catheptic enzymes.

#### Determination of chemical characteristics

Moisture, fat and nitrogen of each sample were determined in triplicate by using the procedures described in AOAC (1965). The protein content was calculated by multiplying percent nitrogen by the factor 6.25.

Sodium chloride was determined in duplicate by adapting the potentiometric titration method of Glasstone (1946) as described by Graham and Blumer (1972).

The pH of each sample was determined by

weighing 20g of muscle tissue, diluting with deionized water, grinding finely in a Waring Blendor for 5 min and the resulting slurry measured with a pH meter equipped with glass electrode.

A recovery study using a mixture of standard amino acids was lyophilized and stored at  $4^{\circ}\text{C}$ . A portion of this standard was then run at each of the three aging time periods (2, 4, 6 months) and served as a guide to the efficiency of the extraction procedure described below.

#### Free amino acid analysis

The ion-exchange column was prepared by using the method described by McCain et al. (1968) with minor modification. A 0.02N HCl solution replaced the 1N HCl for washing the Dowex  $2 \times 10$  resin which was then followed by rinsing several times with deionized water. Acid washing of Rexyn 210 (C1) proved to be unnecessary.

The deproteinization method of Tallan et al. (1954) as modified by McCain et al. (1968) was used to remove the proteins. 5g of minced sample were placed in a Lourdes mixer cup (Lourdes Instrument Corp., Brooklyn, N.Y.) and homogenized 5 min with approximately 75–100 ml of 1% picric acid. The mixture was kept cool by placing the blender cup in a container of ice water. The slurry was transferred quantitatively to a centrifuge bottle and knives and cup rinsed carefully with 25 ml of distilled water. The mixture was then centrifuged at 3,500–4,000 rpm ( $2,000\text{--}2,520 \times g$ ) at  $4^{\circ}\text{C}$  for 25 min. The resulting supernatant was decanted and passed through a column of Dowex  $2 \times 10$  ion-exchange resin-chloride (Bio-Rad Laboratory) or Rexyn 201 (C1) (Fisher Scientific Co.) to remove the excess picric acid. Either resin proved satisfactory. The walls of the column and resin were washed with 25–30 ml of 0.02N HCl. The effluent together with the rinsings were frozen, lyophilized and stored at  $4^{\circ}\text{C}$  until used for amino acid analysis.

A weighed amount of freeze-dried sample was dissolved in a citrate buffer of  $\text{pH } 2.20 \pm 0.03$  to give a concentration of approximately 1 mg amino acid (AA) per ml after dilution. Filtration of diluted samples through Gelman millipore filters (ca  $0.20\mu$  pore size) was done to avoid contamination of the analyzer columns. The free amino acids were analyzed with a Beckman Model 116 and Model 119.

The free amino acids from each sample were compared with amino acid standards run under the same experimental conditions.

#### Isolation and determination of catheptic activity from hams aged at different periods

Finely ground, thawed samples were placed

Table 1—Statistical analysis and values for % moisture, % fat, % protein, % NaCl, pH and enzymatic activities of aged cured hams

Variable	Lot mean <sup>a</sup> Frozen at -29°C				Std error of lot $\bar{x}$	Average change per mo for all lots	Std error of change	Error mean square	Test of lots F <sub>1</sub> <sup>b</sup>	Test of regres- sion F <sub>2</sub> <sup>c</sup>
	1	2	3	4						
	Fresh chilled then cured	Stored 2 days then cured	Stored 2 mo then cured	Stored 1-3 yr ( $\bar{x}$ =2.3) then cured						
% Moisture	63.62	60.76	60.21	61.36	1.79	-1.90	0.55	9.58	0.16	12.04*
% Fat	6.80	9.42	8.70	8.47	1.33	0.58	0.41	5.34	0.27	2.05
% Protein	21.77	22.68	24.17	22.75	0.70	0.83	0.21	1.45	0.89	15.03*
% Salt	3.89	4.22	4.38	4.09	0.25	0.31	0.08	0.19	1.21	16.61*
pH	6.45	6.46	6.23	6.38	0.09	-0.01	0.03	0.02	0.17	0.24
Enzyme activity <sup>d</sup>										
KCl crude <sup>e</sup> extract	0.93	1.00	1.07	1.05	0.09	-0.08 <sup>f</sup>	0.03	0.03	0.18	7.40
Acid treated <sup>e</sup> crude extract	1.10	1.12	1.08	1.25	0.06	-0.12 <sup>f</sup>	0.02	0.01	1.39	34.33**

<sup>a</sup> Means for values obtained after aging 2, 4 and 6 months. All analyses were done in duplicate or triplicate.

<sup>b</sup> Test for differences between lots with respect to change (Test of lots);  $F_1 > 6.60$  = significance at 5% level.

<sup>c</sup> Test of significance of avg trend by regression at 5% level\* ( $F_2 > 7.71$ ); at 1% level\*\* ( $F_2 > 21.20$ )

<sup>d</sup> Data in log values to linearize the relationship and stabilize the variance

<sup>e</sup> Enzyme activity units expressed as antilogarithm of the lot means (unit per mg protein after 4 hr incubation). Protein content determined for each extract by determination of nitrogen by micro-Kjeldahl (N X 6.25).

<sup>f</sup> The "Average change per month" on the log scale is not a percentage change (8 and 12% decrease).

in a Waring Blendor and homogenized with 2% KCl (1:3 w/v) for 3 min. Homogenization was performed in several batches depending on the amount of sample used. The homogenate was allowed to stand overnight at 4°C and then centrifuged between 8,000–9,000 rpm (10,400–13,200 X G) at the same temperature. The supernatant was referred to as the potassium chloride extract. A given volume of KCl extract was dialyzed against deionized water overnight at 4°C.

The acid extract was prepared by using measured quantities of the above extract and adding 1N HCl with continuous stirring to a final pH of 3.8. The mixture was stirred for an additional 10 min and then centrifuged at 13,200 X G for 15 min. The filtrate was adjusted to pH 6.8 with 1N KOH and again centrifuged as above and is referred to as the acid treated crude extract. Total protein and enzymatic activity of the acid extract was determined.

Enzyme extracts from each ham were prepared in duplicate or more and a micro-Kjeldahl determination of nitrogen was made on the KCl and acid treated extracts for the purpose of calculating the amount of "enzyme protein" (N X 6.25) present in each. The average enzyme activity of duplicate enzyme protein extracts was calculated and determined as shown below.

Enzymatic activity was determined by following the modified procedure of Anson (1938) for cathepsin D assay. The reaction mixture consisted of 2.5% denatured hemoglobin in 0.2N acetate buffer (pH 3.8), 0.066N sodium acetate buffer (pH 3.8), and enzyme source (1:4:1, v/v/v). After incubation for 4 hr at 45°C the reaction was stopped by the addition of 10% TCA (volume used was twice that of the reaction mixture). Controls were prepared in the same manner except that the substrate was added after incubation. Controls and samples

were held overnight at 4°C and centrifuged at 3,600 rpm for 15 min. TCA soluble filtrates were then read at 274 nm in a Shimadzu or Cary 15 spectrophotometer. The increase in optical density indicated the enzyme activity in 4 hr per volume of enzyme extract used and was expressed as units of enzyme activity in 4 hr per mg of protein.

## RESULTS & DISCUSSION

### Chemical characteristics

A comparison of the effects of pre-curing treatments (lots) are shown in Table 1. It should be noted that each value presented is an average of the values obtained after the hams were aged 2, 4 and 6 months. The fresh chilled and cured hams lost less moisture than those which were frozen and thawed prior to curing. However, the length of freezing time did not have an appreciable effect on moisture losses. As may be observed in Table 1, the test of average trend by regression indicates that the losses for treatments were different for percentage moisture, protein and NaCl. The differences are due to the greater moisture retention of fresh chilled and cured hams. However, no important difference among lots of hams was found in the rate of change of moisture loss with length of aging period (Table 1). It has been reported previously that freezing meat disrupts the muscle structure such that moisture loss in the thawed stored product is increased over that of fresh stored meat (Lawrie, 1966). However, Graham and

Blumer (1972) did not find much difference in moisture losses of hams fresh-cured and hams frozen, thawed and then cured.

### Catheptic activities of fresh and cured hams

The lot means were transformed to a log scale to linearize the relationship and stabilize the variance between enzyme activity and sampling periods. Table 1 shows an 8 and 12% average decrease in enzyme activity per month of aging for the potassium chloride and acid treated extracts, respectively. It should be noted that these extracts represent successive steps in the purification of the enzymes. Thus, higher enzyme activity was found for the acid treated crude extract than for the KCl extract. The acid extract enzyme activity was highest in the cured hams which had been frozen 1–3 yr ( $\bar{x}$  = 2.3). Thus, it may be safely predicted that hams may be frozen for long periods prior to curing without affecting catheptic enzyme activity.

The individual lot regression for each of the factors measured is given in Table 2. The rate of change is shown for each lot over the 4 months (2–6) aging. It should be understood that the initial analytical measurements began after hams had been aged 2 months. Thus, the rate of change per month for any factor may be calculated by dividing each value shown in Table 2 by four. However, there were no appreciable differences found among the four lots.

The data presented in Table 3 are part of those obtained during the isolation, purification and characterization of the catheptic enzymes from the fresh and cured hams. The cured hams are the same as those shown in Table 1, but a fresh ham was analyzed at each sampling period for comparative reasons as mentioned previously. Catheptic activity in the KCl extracts was similar up to 4 months but it may be noted in Table 3 that it decreased appreciably at 6 months. Acid extract enzyme activity values were highest after 2 months aging and decreased at both 4 and 6 months. No significant differences were noted within aging periods. It may be noted in Table 3 that although acid extract enzyme activity values are higher than for KCl extract, the trend in enzyme activity was similar for both extracts at each sampling period including the fresh hams.

The decrease in catheptic activity with aging time of cured hams may be attributed to high salt concentration and possibly to decreased water-activity. Although the total amount of NaCl remained constant, the concentration increased as a result of continuous reduction in moisture content; therefore, some of the catheptic enzymes were precipitated or denatured. The crude enzyme extracts obtained in this study when in-

Table 2—Individual lot regression for proximate analysis, NaCl, pH and enzyme activity<sup>a,b</sup>

Variable	Lot 1	Lot 2	Lot 3	Lot 4
Moisture	-5.82	-7.49	-10.01	-7.05
Fat	3.79	2.88	2.82	0.13
Protein	1.94	1.95	5.11	4.22
NaCl	0.26	1.56	1.76	1.43
pH	-0.15	0.00	0.04	-0.10
Enzyme activity <sup>c</sup>				
KCl crude extract	-0.24	-0.40	-0.40	-0.22
Acid treated crude extract	-0.54	-0.19	-0.62	-0.52

<sup>a</sup> The rate of change was calculated for each lot over the 4 month aging period (2-6). The rate of change per month may be obtained by dividing each value above by 4.

<sup>b</sup> All analyses were done in duplicate or triplicate.

<sup>c</sup> A log transformation was used to linearize the relationship. Thus, on the log scale the above values are in percentages.

Table 3—A comparison of the proteolytic activities of the potassium chloride and acid treated extracts of fresh and cured hams of different aging periods

Treatment	Specific activity <sup>a</sup> × 10 <sup>6</sup>			
	Fresh hams	Cured hams <sup>b</sup>		
		2 months <sup>c</sup>	4 months <sup>c</sup>	6 months <sup>c</sup>
Potassium chloride crude extract	12.43	13.70	13.06	6.72
Acid treated Crude extract	16.76	25.85	12.29	9.04

<sup>a</sup> The specific activity is expressed as units per mg of enzyme protein per g of muscle tissue.

<sup>b</sup> Cured hams are the same as Table 1.

<sup>c</sup> Average of analyses of four lots of hams.

Table 4—Quantity of free amino acids and statistical analysis of hams by treatments (lots)

Variables	Lot means <sup>a,b</sup>				Std error of lot $\bar{x}$	Avg change per mo all lots	Std error of change	Error mean square	Test of lots $F_1$ <sup>f</sup>	Test regression $F_1$ <sup>g</sup>
	1	2	3	4						
	Fresh chilled then cured	Frozen storage before cure		1-3 yr ( $\bar{x}$ =2.3)						
Alanine	159.8 <sup>c</sup>	185.0 <sup>d</sup>	175.9 <sup>e</sup>	238.2 <sup>c,d,e</sup>	9.9	47.5	3.0	295	2.9	244.1**
Arginine	29.8	20.3	34.3	47.6	9.5	1.7	2.9	273	0.2	0.3
Aspartate	34.3 <sup>c</sup>	19.9 <sup>c,d</sup>	32.4	36.3 <sup>d</sup>	1.7	3.4	0.0	9	2.2	39.0**
Cystine	0.0	0.0	0.0	0.0	—	—	—	—	—	—
Glutamate	101.2 <sup>c</sup>	95.8 <sup>d</sup>	102.5 <sup>e</sup>	129.6 <sup>c,d,e</sup>	7.7	23.9	2.4	177	0.4	102.9**
Glycine	88.5 <sup>c</sup>	94.5 <sup>d</sup>	98.1	116.1 <sup>c,d</sup>	5.9	20.0	1.8	104	1.0	122.7**
Histidine	99.2	86.6 <sup>c</sup>	103.6	113.2 <sup>c</sup>	6.0	6.3	1.8	107	0.3	11.8*
Isoleucine	63.6	54.3 <sup>c</sup>	60.5	75.5 <sup>c</sup>	3.1	13.4	1.0	29	1.7	194.2**
Leucine	72.0 <sup>c,d</sup>	92.0 <sup>e</sup>	104.3 <sup>c</sup>	128.2 <sup>d,e</sup>	8.8	22.5	2.7	230	0.7	70.4**
Lysine	124.8	99.3 <sup>c</sup>	123.3	165.0 <sup>c</sup>	17.0	27.6	5.2	864	0.6	28.1**
Methionine	23.9 <sup>c</sup>	25.4 <sup>d</sup>	29.7	36.2 <sup>c,d</sup>	2.2	7.3	0.7	15	1.8	110.6**
Phenylalanine	38.5	41.7	49.4	49.4	8.4	9.4	2.6	212	0.2	13.4*
Proline	67.3	62.2	60.3	83.7	7.5	16.8	2.3	170	0.2	53.4**
Serine	75.9 <sup>c</sup>	61.6 <sup>d</sup>	74.5	101.2 <sup>c,d</sup>	5.2	8.1	1.6	83	0.6	25.1**
Threonine	60.6 <sup>c</sup>	54.2 <sup>d</sup>	62.7	80.8 <sup>c,d</sup>	5.6	10.7	1.7	96	0.4	38.0**
Tryptophan	0.0	0.0	0.0	0.0	—	—	—	—	—	—
Tyrosine	26.1	20.8 <sup>c</sup>	28.0	33.3 <sup>c</sup>	2.4	4.6	0.7	17	0.5	38.7**
Valine	73.6	74.9	85.5	94.8	8.4	16.2	2.6	212	0.2	39.8**
Ammonia	160.1	235.5	218.7	202.2	49.7	2.7	15.2	7418	0.5	0.0

<sup>a</sup> Represents the means for values obtained after 2, 4 and 6 months aging. All analyses were done in duplicate for each ham sample.

<sup>b</sup> Micromoles of amino acid per g of lyophilized sample

<sup>c,d,e</sup> All mean values with the same superscripts are different from each other.

<sup>f</sup> Test for differences between lots with respect to change (Tests of lots);  $F_1 > 6.60$  = significance at 5% level.

<sup>g</sup> Test of significance of avg trend by regression at 5% level\* ( $F_2 > 7.71$ ); at 1% level\*\* ( $F_2 > 21.20$ ).

Table 5—Relative amounts of free amino acids of fresh and cured hams aged at different periods

Amino acids	Fresh hams <sup>a</sup> × 10 <sup>2</sup>	Cured hams <sup>a</sup> × 10 <sup>2</sup>		
		2 months <sup>b</sup>	4 months <sup>b</sup>	6 months <sup>b</sup>
Alanine	11.0	9.3	19.4	28.3
Arginine	1.1	2.5	4.2	3.2
Aspartic acid	1.2	2.3	3.3	3.6
Cystine	0.4	0.0	0.7	0.8
Glutamic acid	2.2	5.8	11.0	15.4
Glycine	4.6	5.9	10.0	13.9
Histidine	25.2	8.8	10.4	11.1
Isoleucine	0.9	3.1	6.3	8.7
Leucine	1.9	5.7	9.4	14.7
Lysine	2.3	6.3	14.8	17.3
Methionine	0.7	1.3	3.0	4.3
Phenylalanine	0.9	2.8	4.2	6.5
Proline	1.4	3.2	7.4	9.9
Serine	3.5	6.3	7.8	9.5
Threonine	1.6	4.0	7.0	8.3
Tryptophan	0.6	0.0	0.0	0.8
Tyrosine	0.7	1.7	2.9	3.5
Valine	2.1	4.8	8.6	11.3

<sup>a</sup> Micromoles of amino acid per mg of lyophilized sample<sup>b</sup> Average of four lots of hams

Table 6—Recovery of standard mixture of free amino acids

Amino acids	Percent recovery		
	I	II	Average
Alanine	98	100	99
Arginine	94	92	93
Aspartic acid	100	100	100
Cystine	88	98	93
Glutamic acid	98	98	98
Glycine	100	102	101
Histidine	102	100	101
Isoleucine	106	102	104
Leucine	104	104	104
Lysine	100	106	103
Methionine	8	6	7
Phenylalanine	100	100	100
Proline	94	94	94
Serine	100	98	99
Threonine	104	100	102
Tryptophan	12	6	9
Tyrosine	100	98	99
Valine	104	106	105
Norleucine	102	98	100

cubated with increasing levels of NaCl decreased proportionally in enzyme activity, but the data are not presented in this paper. Other workers, Reddi et al. (1972), have shown previously that NaCl inactivated the cathepsin D activity of fish muscle and that it affected the enzyme per se rather than the substrate. Also, Deng and Lillard (1973) showed that high levels of NaCl decreased the activity of porcine muscle cathepsins.

It is obvious that extending the aging period of cured hams for several months has the effect of reducing catheptic

enzyme activity and that holding hams in frozen storage at a low temperature for long periods is not detrimental to specific enzyme activity. Thus, hams may be purchased and stored frozen to hold for curing in the future. This would be advantageous to processors who have freezer-storage facilities and can buy fresh hams at low cost by volume purchase or low market price.

#### Free amino acid production in country-style hams

The values shown for each free amino

acid by lots is an average of the quantity present after hams were aged 2, 4 and 6 months (Table 4). Lot 4, frozen 1–3 yr before curing, contained a greater total quantity of free amino acid than that of any other lot. The quantities for lots 1 through 4 are 1139, 1088, 1214 and 1529  $\mu$ moles of amino acid per gram of lyophilized sample, respectively. These totals are not shown in Table 4, but may be obtained by adding the values shown for each lot. Also, a comparison of lots 1, 2 and 4 indicates that the latter contained appreciably greater amounts of many of the individual free amino acids. This is in agreement with the data presented in Table 1 where enzyme activity for the acid treated extract was shown to be greatest for lot 4. These results may have been due to greater accessibility between enzyme and enzyme active sites during aging of hams and perhaps to the greater ease of recovery of the enzymes from hams frozen and stored for the longest time period prior to curing. This explanation could account for the greater quantity of free amino acid produced during aging of lot 4 hams. However, Piskarev and Dibiraulaev (1971) reported that the lower the freezing temperature the greater the accumulation of free amino acid. Thus, if this is the case there may have been a greater quantity of free amino acids present in this lot prior to cure. The intermediate length of frozen storage pre-cure of lot 3 hams resulted in the intermediate amounts of free amino acids present after aging (Table 4). Only the amino acids arginine, cystine and tryptophan and ammonia do not show an appreciable change over the time periods.

The results presented show the association between pre-curing treatment, specific enzyme activity and free amino acid production of country-style hams.

The amount of free amino acids present in fresh hams and in dry cured hams with time of aging may be observed in Table 5. The data show that histidine is the free amino acid present in greatest quantity in fresh hams and alanine in cured hams at each of the three aging periods. Cystine and tryptophan were observed within some of these hams, but their presence is not consistent and is negligible in quantity. This inconsistency may be due to the low percentage recovery of tryptophan, but does not explain the low recovery of cystine (Table 6). However, methionine recovery was also low, but was measurable in each lot of hams. McCain et al. (1968) did not find cystine in any ham samples analyzed. The other amino acids found in the hams were almost completely recovered from the standard solution used to illustrate the efficiency of recovery by this procedure.

The high content of free histidine in



the fresh hams (Table 5) could arise from the water soluble pigments, myoglobin and hemoglobin, of the sarcoplasmic proteins. This result is supported by Knipfel et al. (1969) who also reported a high free histidine content in fresh hams. The free amino acids reported in this study showed similar patterns to those found by Macy et al. (1964a, b) and McCain et al. (1968).

The information obtained in this study suggests that catheptic enzymes are involved in the production of free amino acids in country-style hams. A more detailed presentation of the extraction, purification and characterization methods of the catheptic enzymes will be published elsewhere.

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## FACTORS RESPONSIBLE FOR WHITE FILM FORMATION ON CUT SURFACES OF DRY-CURED HAMS

### INTRODUCTION

COUNTRY-STYLE HAM is an important agricultural product of many southeastern states. The distinctive aged flavor makes this product unique and several studies have shown the relationship between aged flavor and specific chemical compounds (Blumer, 1954; Brady et al., 1949; Craig et al., 1964; McCain et al., 1968; Ockerman et al., 1964).

This product is marketed sliced in vacuum packages which extends shelf life, preserves aged flavor, and makes the product more appealing to the consumer. One of the most troublesome and costly problems encountered in marketing sliced country-style ham is the development of a white film on the cut surfaces. It is estimated that about 5% of the ham marketed in this manner may be returned to the producer from retail outlets because of surface film formation.

Previous work in this laboratory revealed that the film was not microbial, and its formation is independent of temperatures from 4–26°C. It is known that the film develops mainly on the semimembranosus, semitendinosus and biceps femoris muscles of ham and less frequently on other muscles. Furthermore, the film does not necessarily form on both hams of a pair, and it may be observed within 24 hr after slicing.

This study was undertaken to determine the composition of the film and the factors involved in film formation.

### EXPERIMENTAL

SAMPLES of country-style ham were procured either from commercial producers or processed from fresh hams in the North Carolina State University Meats Laboratory. Those processed in the laboratory and by commercial processors were cured and aged similar to methods described by McCain et al. (1968), except they were not smoked. Presently, nearly all the commercially produced country-style hams are not smoked after curing. Hams were cured with 31g per 0.45 kg of a dry curing mixture consisting of 3.63 kg NaCl, 0.91 kg white sucrose, 71g NaNO<sub>3</sub> or KNO<sub>3</sub> and 14g of NaNO<sub>2</sub>. The hams remained in cure for about 2 days/0.45 kg of ham at 4°C. They were then removed from cure, placed in stockinettes and hung in a room at 10°C for 20–30 days to effect salt equalization. Thereafter, the hams were aged at 29 ±

3°C for 30 or more days to develop the proper flavor. Hams were then sliced and packaged under 16–20 inches of Hg and packaged under vacuum in gas tight polyester, polyvinylidene chloride, vinylidene copolymer (Saran and Cryovac) transparent pouches.

Some of the commercial samples had already developed white film on the surfaces of the packaged slices when received while other slices were freshly packed and then stored at 4°C to permit film formation.

Samples of surface film were obtained by carefully scraping the surfaces of the film covered slices. The film was then extracted with diethyl ether to remove fat and then a small amount was dissolved (< 1.0 mg/ml) in an ammonium hydroxide-acetate (0.54M acetic acid, 0.10M NH<sub>4</sub>OH, pH 4.0) buffer. These solutions were concentrated threefold on a rotary evaporator at 40°C. Crystals precipitated from these solutions when they were stored at 4°C and were later identified by ultraviolet spectroscopy (in ammonium hydroxide-acetate buffer, pH 4.0; 200–315 nm, Bausch and Lomb Spectronic 505 spectrophotometer) and amino acid analysis (Beckman Model 116 Amino Acid Analyzer).

#### Gel chromatography

Several subsequent samples of films prepared by the methods described above were placed in the ammonium hydroxide-acetate buffer (pH 4.0) and passed through a 0.9 × 41 cm column of Bio-Gel P-2, 50–100 mesh, (Bio-Rad Laboratories, Richmond, Calif.); (V<sub>0</sub>, 10.0–10.2 ml; flow rate 0.93 ml/min) in an effort to determine if the film contained peptides. The buffer was used as eluant and 0.8–1.1 ml fractions were collected. A biuret test was performed on each of these fractions.

Three peptide fractions isolated were subjected to high voltage electrophoresis (2,500v, 60 ma for 1–3 hr) on a Shandon Model L24 electrophorator (Shandon Scientific Co.; Willesden, London) using pyridine-acetic acid buffer (pH 3.5).

The ultraviolet spectra of two of these peptide fractions from gel chromatography were also determined on a Bausch and Lomb Spectronic 505 recording spectrophotometer and compared to that of L-tyrosine, L-tyrosyl-L-tyrosine, L-tyrosyl-L-phenylalanine and L-tyrosylglycylglycine.

#### Film induction experiments

A suspension of the crystals was used to induce film formation on 7 mm thick film-free slices of country-style ham. Approximately 1–2 ml of suspension was applied to the slice surfaces with a glass rod and the surface then dried with a heat gun. This application was repeated after which slices were covered with a plastic film and stored at 3°C along with con-

trols. Observations on film formation were made after 5, 7, 10 and 13 days storage. Similar experiments were performed using aqueous solutions of glycerol, L-tyrosine, L-tyrosylglycylglycine, L-tyrosyl-L-phenylalanine, L-tyrosyl-L-tyrosine, L-aspartic acid, L-alanine, L-cysteine, L-glutamic acid, L-histidine, L-isoleucine, L-leucine, L-phenylalanine, DL-proline, serine, L-taurine, L-threonine, L-tryptophan and L-valine.

Diethyl ether solutions of palmitate, stearate, oleate, linoleate, linolenate and a mixture of free fatty acids from lard were also used to test their effect on film formation. Finally, combinations of L-tyrosine and the free fatty acid mixture were applied to ham surfaces. Film formation was determined subjectively using a one to seven point scale ranging from none to very heavy.

#### Tyrosine levels in ham with film

This experiment was undertaken to determine the levels of free tyrosine in commercially processed country-style ham with and without surface film.

Three samples of sliced ham with varying amounts of film were procured, removed from their vacuum packages and each sample randomly divided into two groups, giving a total of six groups. External fat and knuckle muscles (vastus lateralis, rectus femoris, vastus intermedius and vastus medialis) were removed from each slice. Remaining were the muscles of the cushion (biceps femoris, gracilis, semimembranosus and semitendinosus) on which the film was most often observed. After recording the amount of film on the trimmed slices, one group of slices from each sample was scraped free of film, while the other group was left intact. The film removed from these three groups was retained for free amino acid analysis. Ham samples from each of the six groups were homogenized by grinding in a Waring Blendor at low speed (17,000 rpm) for 2 min or until homogeneous. Determinations were made on fat, moisture, salt, free amino acids and pH according to methods described below.

A similar experiment was conducted using slices where no film had formed on the muscles of the knuckle, but where film was present on the muscles of the cushion. Three samples were trimmed free of external fat and each sample divided into two groups by separating the knuckle from the cushion. The resulting six groups were analyzed as in the preceding experiment, except that the film was not analyzed separately since it was allowed to remain on the three groups of cushion muscle samples.

#### Analytical procedures

The percent fat and moisture were deter-

mined on duplicate homogenized samples according to AOAC (1965). Duplicate samples agreed within  $\pm 0.25\%$ .

Sodium chloride content was determined to  $\pm 0.25\%$  accuracy on duplicate homogenized samples by the method of Glasstone (1946) as adapted by Graham and Blumer (1972).

The pH of samples was determined by placing calomel and reference electrodes firmly against the surface of the slice which had been moistened with distilled water. The pH was measured ( $\pm 0.05$ ) at three different locations on the slice and these values averaged. The pH values at the three locations agreed within  $\pm 0.1$ . Surface pH was used since the slices were to be preserved to observe film formation. However, the homogenate of ham slices was found to have nearly the same pH as the surface measured slice.

Free amino acids were extracted and the sample deproteinized by the method of Tallan et al. (1954) as modified by McCain et al. (1968). The protein-free extracts were lyophilized (Virtis Freezemobile, Virtis Inc., Gardiner, N.Y.) and stored at  $2^\circ\text{C}$  until used (1–5 days). Amino acids were measured using a Beckman Model 119 Automatic Amino Acid Analyzer. The preparation of the buffer solutions and the complete operating instructions were those furnished by the manufacturer. A recovery study was conducted to insure precision and accuracy. Recovery rates were greater than 93% for all amino acids except methionine and tryptophan, which are not reported here.

## RESULTS & DISCUSSION

### Recrystallizations and related experiments

The fat free film dissolved in the ammonium hydroxide-acetate buffer precipitated as crystals from solution in as short a period as 24 hr at  $4^\circ\text{C}$ . The crystals were identified by ultraviolet spectroscopy and amino acid analysis. The ultraviolet spectra suggested that the crystals were L-tyrosine (Fig. 1). The peptide fractions from gel chromatography were not identified, although they exhibited a strong broad peak at 275 nm suggesting they contained tyrosine (Fig. 1). None of the peptide fractions had spectra similar to any of the known tyrosine containing peptides determined. Two acid hydrolyzed crystalline samples were found to be 88–94% L-tyrosine by amino acid analysis. The same samples analyzed without hydrolysis were 93 and 97% free L-tyrosine, indicating a high concentration of free L-tyrosine in the film. This result was confirmed later when the amount of L-tyrosine in ham film was quantified. Biuret tests on two crystal samples were negative, as would be expected for free tyrosine.

### High voltage electrophoresis

Peptide fractions from gel chromatography migrated 11.0–12.5 cm towards the cathode at pH 3.5 indicating a positively charged particle. All three samples were homogeneous, suggesting the presence of only one peptide in the film.

Table 1—Composition<sup>a</sup> of ham slices with and without film

Components analyzed	Slices with film	Slices with film removed	L-tyrosine in film
% Moisture	52.69	53.24	
% Fat	13.09	11.50	
% Sodium chloride	7.46	7.74	
pH	5.7	5.7	
Total free amino acids <sup>b</sup>	264.0	281.2	
Acidic and neutral free amino acids	200.7	215.5	
Basic free amino acids	63.3	65.7	
Alanine	30.0	33.6	
Arginine	7.8	5.1	
Aspartic acid	9.7	11.0	
Cysteine	0.5	0.8	
Glutamic acid	24.6	28.1	
Glycine	18.0	19.5	
Histidine	20.5	21.4	
Isoleucine	12.4	8.7	
Leucine	22.9	25.4	
Lysine	35.0	39.2	
Phenylalanine	9.9	10.5	
Proline	11.5	13.6	
Serine	21.0	22.0	
Threonine	16.2	18.4	
Tyrosine	7.9	6.1	287.3 <sup>c</sup>
Valine	16.2	17.8	

<sup>a</sup> Average of three samples

<sup>b</sup> All amino acid values reported as  $\mu\text{moles/g}$  of ham

<sup>c</sup>  $\mu\text{moles/g}$  of film

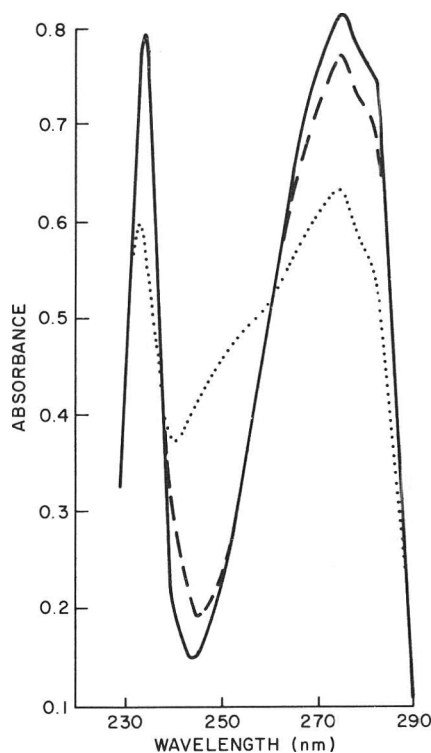


Fig. 1—Ultraviolet spectra of 0.2 mg/ml L-tyrosine (—), 0.2 mg/ml recrystallized material (---) and peptide fraction from gel chromatography (· · ·).

### Film induction experiments

Ham slices treated with a suspension of the crystals produced slight to moderate film after 10 days storage at  $4^\circ\text{C}$  while 75% of control samples showed no film and the remainder only slight film. Slices treated with a 5 mg/ml solution of L-tyrosine also produced film while those treated with tyrosine containing peptides, and 14 other free amino acids produced no film. Individual free fatty acids from lard enhanced film formation when applied to slices together with L-tyrosine. However, this effect was not reproducible. Nevertheless, free fatty acid enhancement of film formation does seem plausible due to the similar hydrophobic properties of L-tyrosine and free fatty acids. Blumer (1954) found an average of 2.5% free fatty acids in country-style ham aged 1 month. The external fat is much higher in free fatty acids as compared to internal fat (Cecil and Woodroof, 1954) and it is mainly the external fat that is smeared across the ham surface during commercial slicing. Later experiments in which ham slices were coated or immersed in corn oil (low free fatty acid content) showed that triglycerides had no effect on film formation. Similarly, glycerol was found to have no effect.

The results indicate that fat has little or no effect on film formation, with the

Table 2—Composition<sup>a,b</sup> of muscle groups from ham slices with and without film

Components analyzed	Muscle groups		
	Cushion <sup>c</sup>	Knuckle <sup>c</sup>	Cushion <sup>d</sup>
	Amount of film		
	Moderately heavy	None	None
% Moisture	58.00	58.26	58.80
% Fat	7.50	4.70	13.29
% Sodium chloride	6.80	9.10	—
pH	5.7	5.8	—
Total free amino acids <sup>e</sup>	362.4	236.0	—
Acidic & neutral free amino acids	285.0	182.5	—
Basic free amino acids	77.4	53.5	—
Alanine	41.6	27.5	23.7
Arginine	18.1	10.3	4.5
Aspartic acid	13.8	8.1	—
Cysteine	0.8	0.9	—
Glutamic acid	39.0	24.0	11.3
Glycine	16.8	16.1	12.8
Histidine	24.2	17.7	8.0
Isoleucine	18.2	11.3	—
Leucine	32.9	20.2	17.8 <sup>f</sup>
Lysine	35.1	25.5	—
Phenylalanine	13.9	8.6	7.1
Proline	17.0	12.0	6.4
Serine	33.6	18.4	9.1
Threonine	25.0	14.9	6.8
Tyrosine	10.5	5.9	3.0
Valine	21.9	14.6	10.2

<sup>a</sup> Average of three samples

<sup>b</sup> All data are from hams aged approximately 90 days

<sup>c</sup> Muscle groups were from the same ham slices

<sup>d</sup> From McCain (1967)

<sup>e</sup> All amino acid values reported as  $\mu\text{moles/g}$  of ham

<sup>f</sup> Value is total of leucine + isoleucine

The difference in the L-tyrosine content of ham before and after film removal indicates that only a small part of the L-tyrosine present in the ham tissue is incorporated into the surface film; thus, the residual concentration is still above the solubility of tyrosine in the water phase of the ham. Perhaps a small reduction in the amount of free L-tyrosine released by catheptic action prevents film formation, since the occurrence of film is variable.

L-tyrosine was determined quantitatively in three samples of ham film and the average concentration was found to be  $287.3 \mu\text{moles/g}$  of film or 5.2% by weight (Table 1). This percentage is probably below the real value because the tyrosine crystals are so small that they cannot be removed from the surface without removing other surface material, especially fat. Nevertheless, the concentration found in the film was 47 times higher than in the tissue from which it was removed (Table 1). A complete amino acid analysis of the film showed that in addition to tyrosine, the following amino acids were present: alanine, glutamate, glycine, histidine, isoleucine, leucine, lysine, proline, phenylalanine, serine, taurine, threonine and valine.

#### Free amino acids in muscles with and without film

The second experiment was conducted with ham slices where film had formed on the cushion muscles, but not the knuckle muscles. The means from the analyses of these two muscle groups, along with similar data from McCain (1967) are shown in Table 2. As in the first experiment, free amino acids were very high as compared to values obtained by McCain (1967). Values for cushion samples were higher than those of knuckle samples, and were appreciable ( $P < 0.1$ ) for L-arginine, L-aspartic acid, L-leucine, L-lysine, L-phenylalanine, L-serine, and L-tyrosine. Average free L-tyrosine was  $10.5 \mu\text{moles/g}$  in cushion samples (moderately heavy film) and  $5.9 \mu\text{moles/g}$  in knuckle samples (no film), which provides support for the reasoning advanced earlier in regard to the influence of quantity of L-tyrosine on film formation.

The percent intramuscular fat was higher ( $P < 0.05$ ) in the cushion samples than in the knuckle samples and this again raises the question concerning the effect of free fatty acids (Table 1).

Statistical analysis of the data was performed using the analysis of variance procedure described by Snedecor and Cochran (1967). The percent sodium chloride was different ( $P < 0.05$ ) in the cushion samples (Table 2) averaging 6.80 compared to 9.10 in the knuckle samples, possibly depressing the catheptic enzyme activity and therefore, the quantity of amino acids released in the latter muscles.

possible exception that free fatty acids from pork may enhance film formation.

#### Tyrosine levels in ham with film

The results of the experiment where ham slices were analyzed before and after surface film was removed are shown in Table 1. The proximate analyses are typical of commercial country-style ham, but the free amino acids are quite high for ham aged only 30–45 days.

The concentrations of free amino acids, including L-tyrosine were about twice those found by McCain (1967) in similarly treated hams (Table 2). There was little difference between L-tyrosine concentration in the ham with or without film removed (Table 1). The average L-tyrosine content was  $6.1 \mu\text{moles/g}$  in ham with film, which is several times the solubility of L-tyrosine in water at  $23^\circ\text{C}$  ( $2.51 \mu\text{moles/g}$  or  $0.454 \text{ mg/g}$ ). L-tyrosine is probably not soluble to the extent of  $2.51 \mu\text{moles/g}$  in commercial country-

style ham, since the ham has only about 55% moisture and sodium chloride and protein are also dissolved in the water phase. The concentration of sodium chloride in the water phase of country-style ham is about 10% and the solubility of L-tyrosine in this solvent is approximately  $1.91 \mu\text{moles/g}$  or  $0.354 \text{ mg/g}$ . Also the isoelectric point of L-tyrosine, 5.63, is near the pH of country-style ham (5.8–6.2). This clearly indicates a mechanism by which film may be formed since L-tyrosine is present in the ham far above its solubility level in the water phase; therefore, it crystallizes out on the cut surfaces. The results are in agreement with Hunt et al. (1939) who referred to the "white flecks" in aged ham and indicated that they contain a large proportion of tyrosine. However, it should be mentioned that these are localized sparsely distributed deposits on the cut surface of the ham and do not resemble the appearance of the white film indicated in this study.

Melo (1972) found a purified cathepsin preparation (60% ammonium sulfate fraction) from fresh or country-style ham to be inhibited 30 and 70% by 5 and 10% sodium chloride, respectively. Also, Reddi et al., (1972) found similar results in cathepsin isolated from winter flounder. Total free amino acids averaged 362.4  $\mu$ moles/g in cushion muscles and 236.0  $\mu$ moles/g in knuckle muscles, a 35% difference. Furthermore, the production of acidic and neutral free amino acids appeared to be inhibited to a slightly greater extent than were the production of basic free amino acids. Acidic and neutral free amino acids averaged 285.0  $\mu$ moles/g in cushions and 182.5  $\mu$ moles/g in knuckles (36% difference), while basic free amino acids were 77.4 and 53.5  $\mu$ moles/g, respectively (32% difference). Sodium chloride inhibition of catheptic activity could be utilized as a method of preventing the accumulation of free L-tyrosine which results in surface film formation up to a level of organoleptic acceptability. Sodium chloride content would be especially important during aging, when the temperature is favorable to catheptic activity.

Partial characterization of catheptic enzymes in fresh and country-style ham indicated the presence of cathepsin A and D (Melo, 1972; Parrish and Bailey, 1966). Parrish and Bailey (1966) determined cathepsin D to be the major cathepsin present in fresh ham and pork loin, while cathepsin A, B and C activity was only barely detectable. Kazakova et al. (1972) found that cathepsin D from rat liver

cleaves low molecular weight substrates at glycyl-leucine bonds adjacent to aromatic residues. This specificity may explain the large amounts of acidic and neutral free amino acids present in country-style ham, since cathepsin A (an exopeptidase) is restricted in its activity to peptides previously degraded by cathepsin D (Iodice et al., 1966). Basic free amino acids are found to a lesser extent probably because the trypsin-like cathepsin B is very low in ham muscle (Parrish and Bailey, 1966).

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## COMPARISON OF SALAMI SAUSAGE PRODUCED WITH AND WITHOUT ADDITION OF SODIUM NITRITE AND SODIUM NITRATE

### INTRODUCTION

FROM JANUARY 1973 the Norwegian Department of Health (1973) introduced a general ban on the use of nitrite and nitrate as food additives. However, temporary exemptions from this general ban have been given to most meat products, cheese and various semipreserved fish products. At present, the restrictions apply to fresh and frozen frankfurter-type sausages and pork sausage. In Norway, the consumption of fresh sausages of the frankfurter type represents the bulk of meat products used, and it is evident that the present restrictions will reduce the intake of nitrite considerably.

The health authorities have strongly urged the meat industry to reduce the amount of nitrite added to the various products, and the meat industry is expending much effort towards this goal. Work done at this Institute has shown that the amount of nitrite added to sliced meat products used for sandwiches (ham, etc.) could be reduced by 70%, corresponding to 30–40 ppm nitrite initially added, without noticeable organoleptic effects (Høyem et al., 1973).

As to fermented sausage, the use of nitrite and/or nitrate has been used for a long time and is thought to be necessary for specific technological reasons. It is the purpose of this study to investigate the microbiological, rheological and organo-

leptic properties of fermented salami sausage produced with and without the addition of nitrite or nitrate.

### EXPERIMENTAL

PRODUCTION and ripening of the salami sausage was performed at the National Institute of Technology in Oslo, while the microbiological, chemical, rheological and organoleptic tests were done at the Norwegian Food Research Institute.

Standardized raw materials were used and the recipe was as follows:

Beef Standard I	41%
Pork Standard II	31%
Lard	22%
Glucose	0,8%
Spices	0,6%
Salt (NaCl)	3,4%
Na-ascorbate	200ppm
Na-nitrite	Not added, or added at 82 and 164 ppm levels

Altogether seven series were produced: series A 164 ppm nitrite added; series B 82 ppm nitrite added; series C no nitrite added; series D 164 ppm nitrite and 0.5% glucono-delta-lactone (GDL) added; series E no nitrite, but 0.5% GDL added; series F 164 ppm nitrite and starter culture (Duplofermente) added; and series G no nitrite, but starter culture (Duplofermente) added.

Salami-type dry sausage usually contains about 164 ppm nitrite and series A could thus be considered as the control, while series B has

a 50% load and series C is produced without the addition of nitrite at all. GDL is used to some extent in the Norwegian meat industry. To see if GDL influenced the fermentation when nitrite was present, or omitted, series D and E were produced. Series F and G contained starter cultures consisting of micrococci and lactobacilli (Duplofermente, R. Müller & Co., Giessen, Germany). The starter cultures are freeze dried and mixed with a carrier (Addition of from 500,000–1 million micrococci and lactobacilli per gram sausage).

The ground meat was stuffed into fibre casings of 70 mm diameter.

The ripening was carried out according to the following schedule:

Days	Temp °C	RH %
1–2	22	92
3–4	20	88
5–20	18	85
21–34	18	80

All the sausages were smoked in the same chamber with beech-wood. The smoking period was started after 36 hr and lasted for 24 hr. The sausages were coated with wax after 34 days of production. The storage time referred to was from this moment on. The waxed sausages were stored at 20°C for 3 months.

The sausages were tested for microbial, chemical and rheological properties immediately after production and after 1, 2, 4, 7, 14, 21, 34 and 124 (34 + 90) days respectively. The weight loss during ripening was registered.

### Microbiological analysis

Bacterial counts during the aging and smok-

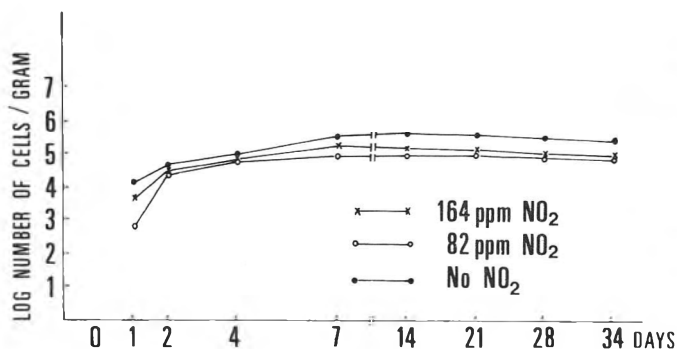


Fig. 1—The incidence of lactic acid bacteria in salami dry sausage with and without the addition of sodium nitrite.

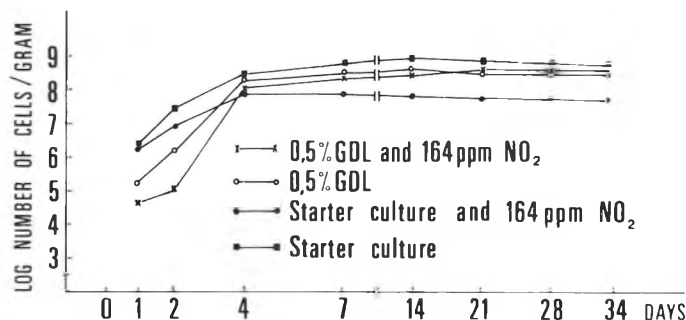


Fig. 2—The incidence of lactic acid bacteria in salami dry sausage produced with different food additives.

ing process were performed as follows: 10-g samples were aseptically removed, 90 ml sterile saline added and then homogenized in a Waring Blendor for 60 sec. Appropriate dilutions were then plated on the media employed.

Lactobacilli were assessed on blood agar (Sandvik, 1963).

King's medium (King et al., 1954) was employed to detect *Pseudomonas*. Incubation was made at 30°C for 48 hr, and counts were made in ultraviolet light. The fluorescent microorganisms were taken as *Pseudomonas*.

Coliform counts were made in Violet Red-Bile Agar (Difco, 1966) after 24 hr at 37°C.

Micrococci and aerobic sporeformers were counted on blood agar (Sandvik, 1963). Incubation took place at 37°C for 24 hr.

#### Water activity

Water activity was measured with a Sina hygroscope (Sina Gerät, model SMT-B). The measurements were done after an equilibration period of 2 hr at 25°C. The water activity was measured in the centers of the sausage.

#### Chemical analysis

Total fat determination was done according to the Gerber method (Pearson, 1963). The determination of nitrite was carried out according to the standard method described by AOAC (1965). Determination of free fatty acids and peroxide numbers were carried out according to Hadorne et al. (1956).

#### Organoleptic analysis

Triangle tests and scoring tests were performed with a test panel of trained laboratory judges selected at the Norwegian Food Research Institute. The sample size was about 50g. Generally two samples were tested at one sitting. In the triangle test 10 panel members were used, and in the scoring test 12. For the scoring test a scale was used where 1 indicated not acceptable quality and 7 was excellent quality (flavor). Red illumination was used in order to exclude the differences in the color of samples with and without nitrite added.

#### Texture measurements

The texture of the salami sausage was measured at various intervals during the ripening period. The equipment used for the tests was an Instron Universal Testing Machine Model TM-SM. A cylindrical plunger 20 mm in diameter was driven at a speed of 10 cm/min, 18 mm into a 20 mm thick salami slice. Under the plunger was a load-sensing cell which

Table 1—Nitrite levels in salami sausage produced with varying amounts of nitrite, GDL and starter culture

Series	Nitrite added (ppm)	Addn of GDL	Addn of starter culture	Amount of nitrite during ripening (ppm)					
				(Days)					
				1	2	4	7	14	28
A	164	—	—	60	45	13	7	5	4
B	82	—	—	24	22	9	4	4	3
C	0	—	—	0	0	0	0	0	0
D	164	+	—	60	41	6	4	4	3
E	0	+	—	0	0	0	0	0	0
F	164	—	+	50	36	6	3	3	2
G	0	—	+	0	0	0	0	0	0

supported the sample to be tested, and the output from the cell was fed into a strip-chart recorder. The maximum force of penetration was registered in grams. Each sample was tested in triplicate, and the average value was used.

## RESULTS

THE DEVELOPMENT of lactic acid bacteria and micrococci during the ripening period is shown in Figures 1–4. These data indicate that nitrite, in the concentrations used, does not influence the growth rate, although there is a tendency for the samples without nitrite to have higher counts 24 and 48 hr after production. Aerobic sporeformers, *Pseudomonas* and coliform bacteria were found in small numbers; no difference was observed between samples with and without nitrite. *Staphylococci* were not found in the present investigation.

The bacterial counts on the day of production must be considered as being satisfactory, and were at the same level for the different series. After 2–4 days the microflora consisted mainly of lactic acid bacteria. An increase in lactobacilli counts could be seen up to the fourth day, and afterwards the bacterial counts tended to level out. GDL did not seem to

inhibit bacterial growth through the first period of incubation. The series with starter cultures added had higher counts of lactic acid bacteria initially; after 4 days of ripening no differences could be seen.

At zero time, the water activity was 0.96, due to the sodium chloride and fat content in the product. Little change in the water activity during the first week of the ripening period was registered (Fig. 5).

In the samples with GDL or starter culture the decrease in  $A_w$  started after 1 wk, while water activity in samples without these additives started to drop after 14 days.

The pH drop in salami dry sausage during the ripening period can be seen in Figure 6. There is a slight increase in pH at the beginning, followed by a fall. The pH in the samples containing GDL fell at a faster rate. The fall in pH of samples with starter culture was more rapid and to a greater extent than in these series without added starter culture.

The fat content on the day of production was 21–22% and when the ripening was finished varied between 31–33%.

Table 1 presents the nitrite level in the sausage at various stages of the ripening

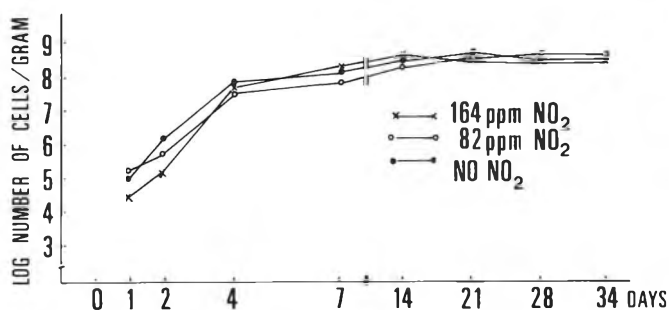


Fig. 3—The incidence of micrococci in salami dry sausage produced with and without the addition of sodium nitrite.

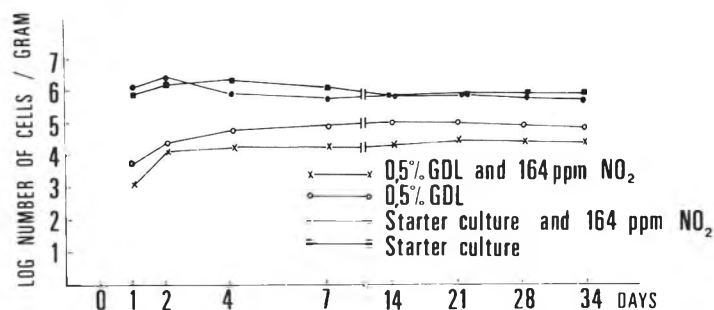


Fig. 4—The incidence of micrococci in salami dry sausage produced with different food additives.

Table 2—Content of free fatty acids and peroxides after 3 months storage at 20°C

Series	Free fatty acids	
	%	Peroxide numbers (meq/kg)
A	13.37	0.75
B	11.49	0.87
C	10.75	1.19
D	16.84	0.40
E	11.94	0.54
F	13.87	0.44
G	13.11	1.10

Table 3—Rheological properties of salami sausage during the ripening process as measured<sup>a</sup> by penetration on Instron Universal Testing Machine (Model TM-SM)

Days after Processing	Series						
	A	B	C	D	E	F	G
2	60	60	70	100	80	60	70
4	65	65	70	120	120	110	220
7	120	130	140	175	170	185	230
14	230	240	230	220	240	265	265
21	380	370	—	340	335	425	440
34	—	435	400	485	450	490	520

<sup>a</sup> Force in grams

process. At the end of the ripening period the nitrite content decreased to 2–4 ppm. No nitrite could be found in the sausages produced without nitrite, except for 1–2 ppm in a 2 mm surface layer. This is probably caused by the smoking process.

Table 2 presents the amounts of free fatty acids and peroxides in the products after storage at 20°C for 3 months. The contents of free fatty acids and peroxides were the same in the different series after 3 months storage.

Table 3 shows the rheological properties of the sausages at various stages during the ripening period. The samples with GDL or starter cultures show a firmer consistency after 2–4 days of ripening compared to the other samples. After 7 days series A to C develop a considerable increase in firmness and after 2 wk the rheological properties, as measured by the method used, tend to be almost equal in the different series.

In Table 4 the results from weight loss during ripening are presented. These data

do not indicate any difference as to the weight loss between the different series.

The results of the organoleptic analyses are presented in Tables 5 and 6. A significant difference in the triangle test was obtained for one test pair only, series A/C, after 3 months storage. A scoring test was performed on this pair, and the results are listed in Table 6. Series A (with nitrite) obtained an average score of 4.7 and series C (without nitrite) obtained 3.2. Series C had an “off flavor” described by the panelists as an “old” taste.

DISCUSSION

THE RESULTS of this investigation indicate that the ripening of the salami sausages takes a normal course without the addition of nitrite GDL or starter culture.

It is well established that lactobacilli and micrococci are of great importance in the ripening of dry sausages. The main objective of the bacteriological investigations was to find out if the addition of nitrite had a detectable effect on the

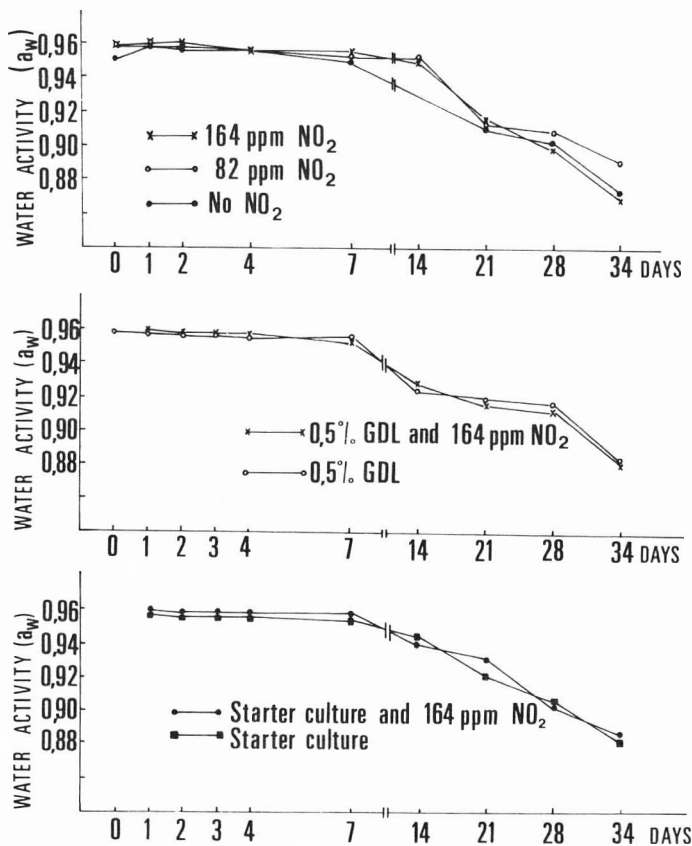


Fig. 5—Water activity in salami dry sausage during the ripening process.

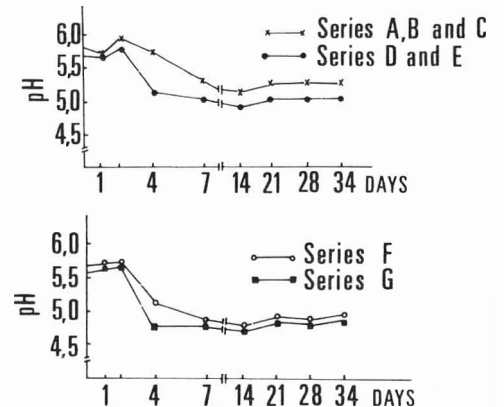


Fig. 6—pH in salami dry sausage during ripening. Altogether seven series were produced (see EXPERIMENTAL for details).



Table 4—Weight loss (in %) in Norwegian salami sausage during the ripening process

Days after Processing	Series						
	A	B	C	D	E	F	G
1	0.75	0.92	0.77	0.96	0.83	0.79	0.88
2	2.2	2.6	2.3	2.0	2.3	2.1	2.4
4	4.8	5.2	4.9	4.6	4.9	4.6	4.7
7	12.1	11.9	12.3	12.1	11.7	10.7	11.6
14	20.0	19.3	19.2	19.7	19.4	19.9	20.2
21	24.8	25.5	23.8	24.6	24.2	25.3	24.7
28	30.0	29.5	29.6	29.7	30.2	31.4	30.4
34	31.2	30.3	30.6	30.8	31.2	32.2	31.4

Table 5—Organoleptic analysis of salami sausage with and without the addition of nitrite, GDL and starter culture

Tested in triangle test	End of ripening period		After 3 months storage at 20°C	
	No. of correct choices	No. of judges	No. of correct choices	No. of judges
Series <sup>a</sup>				
A/C	9	20 NS <sup>b</sup>	9	10 +*
B/C	8	20 NS	5	10 NS
D/E	9	20 NS	6	10 NS
F/G	9	20 NS	5	10 NS

<sup>a</sup> Series A: Contained 164 ppm nitrite; Series B: Contained 82 ppm nitrite; Series C: No nitrite added; Series D: Contained 164 ppm nitrite plus 0.5% GDL; Series E: No nitrite added, but contained 0.5% GDL; Series F: Contained 164 ppm nitrite + starter culture (Duplofermente); Series G: No nitrite added, but contained starter culture (Duplofermente)

<sup>b</sup> NS = Not significant

\* Significant at  $P \times 0.05$

bacterial flora. No positive or negative effects of nitrite could be demonstrated. The microflora was very similar in series with and without nitrite, the only exceptions were the two series where Duplofermente was added. In this case series without nitrite had a higher count of lactobacilli. These results have probably little connection with the addition of sodium nitrite, because similar effects were not observed in the other series. It is possible that the differences were due to the fact that in the series without nitrite lactobacilli better adapted to growth were added. This series was the last one produced, and therefore the Duplofermente was equilibrated for an extra 30 min in 0.1% peptone water. The ripening of dry sausage seems to proceed as well in the series without nitrite as in series with nitrite and that the addition of nitrite in low concentrations has no demonstrable effect on the fermentation of dry sausages.

The water activity in Norwegian salami sausage after ripening varies between 0.84 and 0.89, and in this experiment the water activity after finished ripening was

0.88. The water activity in the centers of the sausages had a high value for a relatively long period of time, but after 2 wk of ripening the water activity started to drop and after 34 days of ripening no difference could be found between the different series. The addition of nitrite, GDL or starter culture did not influence the water activity in the sausages.

The decrease in pH value is mainly a result of microbial activity, but in series where GDL was added, the fall was due

Table 6—Scoring test evaluation of flavor of salami sausage after 3 months storage at 20°C

Tested in Scoring test	Avg of scores <sup>a</sup>
Series A	4.7
Series C	3.2

<sup>a</sup> Averages of scores of 12 judges on a scale of 1 = not acceptable quality of salami; 4 = acceptable quality of salami; and 7 = excellent quality of salami.

to a combined effect of GDL and microbial growth. The addition of nitrite does not seem to influence the fall in pH. This result corresponds to the earlier findings that the series with and without nitrite had a similar microflora.

The nitrite concentrations in the sausages were low at the end of the ripening period. All of the values finally reached a value recorded as 2–4 ppm, which is essentially the same. It is well known that the available amount of nitrite decreases considerably during processing and subsequent fermentation and storage.

The red-colored surface layer in series without nitrite was due to the smoking process. The processing of dry sausage includes rather heavy smoking of the products, and a transmission of nitrite to the surface leads to the formation of nitrosomyoglobin. Wasserman and Talley (1972) reported similar findings.

From the results of the consistency and weight loss measurements, no effect was demonstrated indicating that the addition of nitrite is desirable or necessary from a technological point of view. The fact that the panelists at the end of the ripening period were unable to distinguish between products with and without nitrite is surprising, because earlier investigations have shown a difference in flavor in other products salted with and without nitrite. Cho and Bratzler (1970) found that heat-treated meat salted with and without the addition of nitrite had different flavors. Similar results were obtained by Wassermann and Talley (1972) in organoleptic tests of frankfurters, and by Skjelkvåle et al. (1973) in organoleptic analyses of frankfurters, meat loaf and meat sausage. In this experiment the heavy smoke on the spices may have added sufficient flavor to the non-nitrite samples to prevent distinguishing between nitrite treatments. However, after 3 months of storage a significant difference was found between products with and without the addition of nitrite, and the nitrite-cured products were given the best score.

In this investigation the addition of GDL and starter culture did not seem to be necessary for obtaining a satisfactory course of the fermentation process, but the results of the organoleptic analysis indicate that GDL and starter culture may improve the storage stability of the products when nitrite is omitted.

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## ISOMETRIC TENSION STUDIES ON CHICKEN PECTORALIS MAJOR MUSCLE

### INTRODUCTION

THE USE OF isometric tension measurements to follow the time course of rigor is a relatively new technique. The first studies on bovine and rabbit muscle were reported by Busch et al. (1967) and Jungk et al. (1967). Prior to this time extensibility measurements were used in order to quantitatively follow rigor mortis. Isometric tension measurement provides several unique advantages over extensibility measurements (Busch et al., 1972a), the most important of which is the ability to detect changes which apparently correspond to resolution of rigor as well as rigor onset.

Schmidt et al. (1968) developed a rigorometer for the measurement of both isotonic and isometric tension and using it, studied factors affecting the time course of rigor mortis in porcine muscle (Schmidt et al., 1970a, b). Isometric tension development and decline in turkey muscle has also been reported (Jungk and Marion, 1970).

However, very little information is available on tension development in chicken muscle and on the relationship between tension development, tension decline and tenderness. Therefore the studies reported herein were undertaken to investigate the influence of pH, temperature and ionic environment on isometric tension patterns in chicken Pectoralis major muscle and to determine the relationship of isometric tension parameters to shear values of the cooked muscle.

### MATERIALS & METHODS

BROILER CHICKENS 8–12 wk old were used during these studies. The birds were exsanguinated by an outside neck cut and slaughter struggle was minimized by restraining the birds in metal funnels. Isometric tension patterns were obtained using an E & M 6-channel Physiograph (Narco Bio-Systems Inc., Houston, Texas). Muscle samples for tension measurements were obtained, immediately after slaughter, by cutting the breast skin and excising a 1 cm wide strip of muscle tissue from the anterior portion of the P. major. The strip was cut parallel to the direction of the muscle fibers and care was taken to prevent stretching of the muscle during excision and subsequent strip preparation. A linear regression equation was prepared relating the weight and cross sectional area of

several muscle strips 5 cm in length and 0.1–1.0 cm<sup>2</sup> in cross section. All strips prepared in subsequent studies were cut to 5 cm in length, weighed and the cross-sectional area determined from the regression equation. Most strips used ranged between 0.15–0.25 cm<sup>2</sup> in cross-sectional area.

One end of a muscle strip was clamped in a battery cable clamp (Muellar No. 43B) and the other end was attached to a second clamp which was fixed on a plexiglass rod within a plexiglass cylinder, 9.5 cm in diameter and 20 cm high. The rod was fixed about 4 cm above the chamber bottom in order to permit the use of magnetic stirring. The chamber was filled with enough buffer to cover the muscle strip and top clamp and the muscle strip was attached to an isometric transducer by means of 6 lb test monofilament fishing line tied to the free clamp. Approximately 5.0g/cm<sup>2</sup> tension was applied to each strip to attain a uniform starting condition. The Physiograph was calibrated so that a 1 cm pen deflection was equivalent to 5g tension. Up to six strips could be studied from each bird. The time lapse from exsanguination to attachment of strips to the transducers varied depending on the number of strips prepared from one bird. A maximum of 20 min was required for preparation of six strips from a single bird.

Ideally, isometric tension should be determined on strips suspended in air but it is difficult to prevent surface dehydration from influencing the results. Preliminary trials with

chicken P. major and the results of Busch et al. (1972a) with rabbit psoas muscle established that tension patterns are essentially the same whether strips are suspended in saturated air or in saline buffer. Thus buffers were used throughout our experiments.

Three different buffer systems were studied: distilled water, phosphate buffer pH 7.2, ionic strength 0.15 (Gomori, 1955) and Tris-acetate buffer pH 7.1, ionic strength 0.22 (Goll et al., 1970), referred to henceforth as solution B (Busch et al., 1972a).

The effect of pH on tension pattern was studied using phosphate buffer. Buffers of pH 5.8, 6.3, 6.7 and 7.2 were prepared as described by Gomori (1955). Six broilers were used and four strips were prepared from each bird. Tension measurements were obtained at room temperature.

Calcium chloride, magnesium chloride and ethylenediaminetetraacetic acid (EDTA) were added to pH 7.2 phosphate buffer in order to study their effect on tension pattern. Solutions were prepared to contain 10<sup>-7</sup>, 10<sup>-4</sup> or 10<sup>-3</sup> M calcium; 10<sup>-3</sup> M magnesium; 10<sup>-3</sup> M EDTA; 10<sup>-3</sup> M EDTA + 10<sup>-3</sup> M calcium; and 10<sup>-3</sup> M EDTA + 10<sup>-3</sup> M magnesium. All treatments were run on a paired basis using buffer at pH 7.2 as the control.

The effect of temperature on tension pattern was studied using muscle strips in phosphate buffer pH 7.2 ranging from 0–60°C. Cold temperatures were attained in cold rooms at 2 and 5°C, and 0°C was attained by pre-

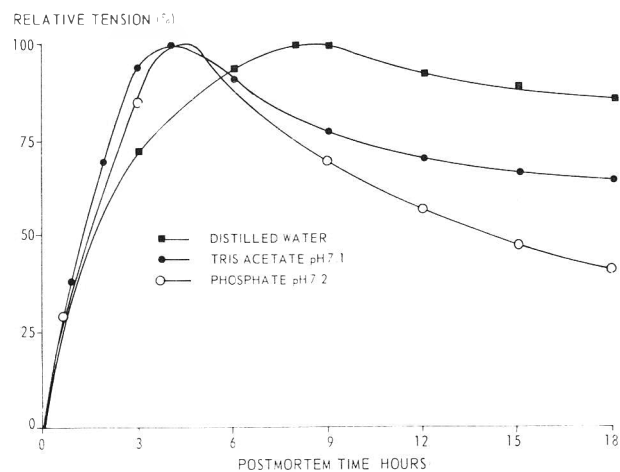


Fig. 1—Effect of buffer on the isometric tension pattern of strips of P. major muscle from chicken broilers. Each point represents the average of six birds, two strips per bird.

cooling buffer in a freezer. Temperatures above room temperature were attained by placing small, 50w aquarium heaters in the buffer chambers and stirring magnetically to maintain even heat distribution. Temperatures studied were 0, 2, 5, 23 (room temperature), 30, 37, 43, 50 and 60°C.

The relationship between tension release and tenderness was studied by measuring the tension pattern and tenderness of 120 birds. Strips for tension measurement were cut from the P. major muscle on one side of the carcass and the breast muscle on the other side was covered with Saran wrap, packed in drained crushed ice and aged on the carcass for 24 hr at 2°C after which tenderness was determined as described by deFremerly and Pool (1960), except 1.5 cm wide strips of cooked muscle were sheared using an Allo-Kramer shear press. A single bladed shear cell, 250 lb ring and 9 cm/min crosshead speed were used for all shear determinations. A minimum of 10 shear/bird were averaged.

## RESULTS & DISCUSSION

THE TENSION PATTERNS obtained in the three buffer systems are shown in Figure 1. Solution B (Tris-acetate) and phosphate buffer produced very similar tension patterns. The main difference between these two systems was in the rate of tension decline. Strips in solution B had declined to about 60% of maximum tension after 16 hr while strips in phosphate buffer had declined to about 45% over the same time period. After 16 hr at room temperature solution B appeared cloudy, whether or not sodium azide was present, suggesting some form of exchange between muscle and buffer. In contrast, the phosphate buffer remained clear up to 24 hr postmortem.

In contrast with buffered systems, the tension of strips in distilled water developed more slowly and showed very little decline. This was probably due to a loss

Table 1—Means and standard errors of the time to maximum tension and amount of tension developed for strips of broiler P. major muscle in 23°C phosphate buffer at four pH levels

pH	Time, hr	Tension, g/cm <sup>2</sup>
7.2	4.50 ± 0.61	40.26 ± 2.80
6.7	3.90 ± 0.63 <sup>a</sup>	34.75 ± 3.92
6.3	3.60 ± 0.39 <sup>a</sup>	27.73 ± 1.51 <sup>b</sup>
5.8	3.33 ± 0.22 <sup>a</sup>	28.15 ± 2.08 <sup>a</sup>

<sup>a</sup> Significantly different from pH 7.2 (P < 0.05)  
<sup>b</sup> P < 0.01

of soluble materials, in particular salt ions, from inside the muscle into the lower ionic strength water.

On the basis of the above findings, phosphate buffer was selected for use in all subsequent tension studies.

### Effect of pH on isometric tension patterns

Tension patterns for muscle strips maintained in phosphate buffer of four different pH levels are shown in Figure 2. The patterns do not differ substantially in rate of tension development or decline but they differ in the amount of tension developed and the time required to reach maximum tension. The true mean values for these parameters cannot be ascertained directly from Figure 2 but are shown in Table 1. Paired comparison t-tests, done by pairing muscle strips run at pH 5, 8, 6.3 and 6.8 individually with the

Table 2—Means and standard errors of time to maximum tension and amount of tension developed for strips of broiler P. major muscle in phosphate buffer at varying temperatures

Temp, °C	Time to maximum tension (min)	Maximum tension (g/cm <sup>2</sup> )
0	3.1 ± 0.2 <sup>a</sup>	65.37 ± 6.5 <sup>a</sup>
2	3.3 ± 0.2 <sup>a</sup>	38.23 ± 3.6 <sup>a</sup>
5	2.2 ± 0.2	17.24 ± 2.3
23	276.7 ± 22.1	46.40 ± 2.0
30	217.8 ± 82.0	49.73 ± 6.5
37	116.5 ± 44.4 <sup>b</sup>	65.46 ± 11.7
43	49.3 ± 15.6 <sup>b</sup>	73.75 ± 9.4 <sup>b</sup>
50	8.1 ± 1.2 <sup>b</sup>	316.89 ± 19.3 <sup>b</sup>
60	0.5 ± — <sup>b</sup>	290.18 ± 29.2 <sup>b</sup>

<sup>a</sup> Significantly different from 5°C (P < 0.01)

<sup>b</sup> Significantly different from 23°C (P < 0.01)

respective control (pH 7.2) strips, showed significant differences in time to maximum tension and the amount of tension developed in each case. Busch et al. (1972a) have reported no change in the amount of tension in rabbit psoas muscle between pH 5.5–7.0 but valid comparisons are difficult to make because of different species, different muscles and different temperatures.

The decreases in time to maximum tension and in amount of tension as pH value decreased are difficult to explain. It has been shown that extracellular pH does not alter intracellular pH but rather changes the selective permeability of the sarcolemma (April et al., 1968; Rome, 1968). If the sarcolemmal permeability of broiler muscle was altered by the lower pH then it is possible that ions or metabolites essential to post mortem tension development were lost through diffusion into the buffer.

### Effect of temperature on isometric tension pattern

During post-slaughter handling poultry carcasses encounter temperatures ranging from 60°C in scald tanks, to 0°C during ice-slush cooling and aging. The effect of temperatures in this range on tension parameters is shown in Table 2.

The data for 0°, 2° and 5°C provide definite evidence of the existence of a cold shortening effect which increases as temperature decreases towards 0°C. deFremerly and Pool (1960) found that the rate of adenosine triphosphate (ATP) decline in postmortem broiler muscle was faster at 0°C than at 10°C and minimal between 10–20°C. Smith et al. (1969) first demonstrated a cold shortening effect in avian muscle. They found that shortening at 0°C was significantly greater than in the 12–18°C range. The present data are in accord with these findings and demonstrate an even more

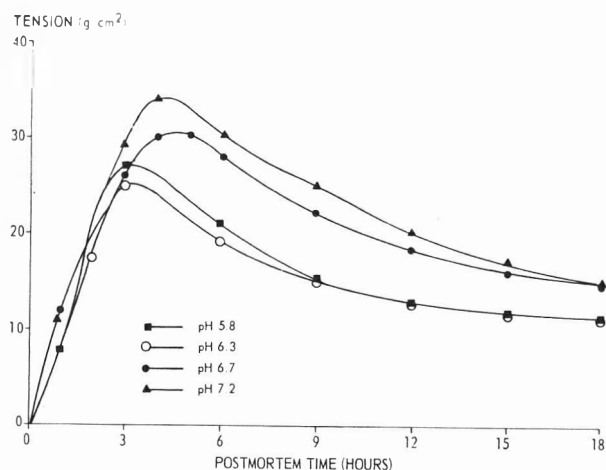


Fig. 2—The influence of pH of phosphate buffer on the isometric tension pattern of strips of P. major muscle from chicken broilers. Each point is the average of six birds.

Table 3—Means and standard errors of time to maximum tension and amount of tension developed for strips of broiler and fowl *P. major* muscle in 23°C phosphate buffer and buffer containing three levels of calcium

	Broilers		Fowl
	Time to max tension (min)	Max tension (g/cm <sup>2</sup> )	Time to max tension (min)
Control	4.18 ± 0.62	51.71 ± 4.32	9.63 ± 0.53
10 <sup>-7</sup> M Ca	3.70 ± 0.72	54.89 ± 7.10	10.17 ± 0.67
10 <sup>-6</sup> M Ca	4.04 ± 0.86	45.61 ± 4.09	9.10 ± 0.71
10 <sup>-3</sup> M Ca	4.06 ± 0.50	36.43 ± 4.63 <sup>a</sup>	10.50 ± 0.62

<sup>a</sup> Significantly different from control ( $P < 0.01$ )

dramatic cold shortening effect than was reported by Smith et al. (1969). These authors found that shortening in broilers was essentially complete after 3 hr whereas in our study tension development was virtually instantaneous and the tension developed had abated within 15–30 min post-maximum tension. The difference between these two sets of data may be partially attributed to the fact that although isometric tension is only a measure of tendency to shorten it can be more accurately determined than actual changes in length. Also, the small muscle strips used in the present study would reach temperature equilibrium much more rapidly than the whole breast muscle used by Smith et al. (1969).

The effect of temperatures from 23–60°C on tension parameters is consistent with the expected increase in rate of glycolysis at higher temperatures. The time required to reach maximum tension declined and the amount of tension increased as temperature increased from 23 to 60°C. Above 37°C the values were significantly different than those at 23°C (Table 2).

The adverse effects of high scalding temperature on poultry tenderness have long been known (Shannon et al., 1957; Klose et al., 1959; Wise and Stadelman, 1959). Wise and Stadelman (1961) suggested two reasons for this effect: firstly, protein denaturation and secondly, an effect caused by holding the carcass at temperatures in excess of normal body temperature. The results obtained in the present study at 50 and 60°C suggest that denaturation would be preceded by a rapid and extensive shortening or contraction of the surface muscle layers. This contracted state could be partly responsible for observed toughening since the association between muscle contraction and tenderness has been well established (Herring et al., 1965; Marsh and Leet, 1966; Howard and Judge, 1968). The above data are also in accord with the findings of Khan (1971) who showed that dephosphorylation of ATP at high temperatures affects the mode or extent

of stiffening of the tissue thus preventing tenderization.

#### Effect of calcium, magnesium and EDTA on isometric tension pattern

The results presented in Table 3 illustrate the effect of three different levels of calcium on tension parameters. The time to maximum tension was not significantly affected in broilers or laying fowl. However, 1 mM calcium did significantly ( $P < 0.01$ ) decrease the amount of tension developed in broiler muscle. A marked difference existed between broilers and fowl in the time required to reach maximum tension. Tension values were not obtained for fowl. The tension release patterns for fowl (hen) and broilers in control and 1 mM calcium buffer are shown in Figure 3. The presence of calcium in the buffer stimulated tension release in both cases. The tension release patterns for 10<sup>-4</sup> and 10<sup>-7</sup> M calcium

levels are not shown but 10<sup>-4</sup> M stimulated tension release significantly whereas 10<sup>-7</sup> M did not differ from the control.

There is a noticeable difference in the rate of tension release between broiler and fowl controls. This difference may relate to the observation that the Z-line in older animals is less labile than that of younger animals (Goll, 1970) and to the extent that percentage release is indicative of tenderness, to the fact that older poultry is less tender than broiler-age poultry (May et al., 1962; Larmond et al., 1971).

The effect of calcium on tension development was not as prominent as on tension release. None of the levels tested affected the time to maximum tension and only 1 mM calcium caused a significant decrease ( $P < 0.01$ ) in the amount of tension developed by broiler muscle. Since calcium initiates contraction *in vivo* (Hasselbach, 1964) the sarcolemma is considered to remain impermeable to the extracellular calcium during most of the pre-rigor period. The observed decrease in the amount of tension developed in the presence of 1 mM calcium suggests that a critical extracellular concentration may have been reached or exceeded thus promoting a more rapid penetration of calcium into the muscle. Busch et al. (1972b) found that 1 mM calcium reduced tension development in rabbit psoas muscle and suggested that a calcium stimulated process caused the loss of isometric tension and that the maximum tension developed represented a balance between tension development and loss of ability to maintain tension at any particu-

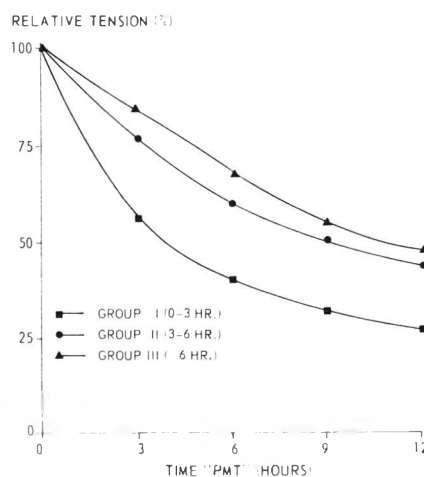


Fig. 3—Effect of 10<sup>-3</sup> M calcium in extracellular phosphate buffer on tension release pattern for *P. major* muscle from chicken broilers and laying hens. Each point is the average of 12 birds. (PMT = post-maximum tension)

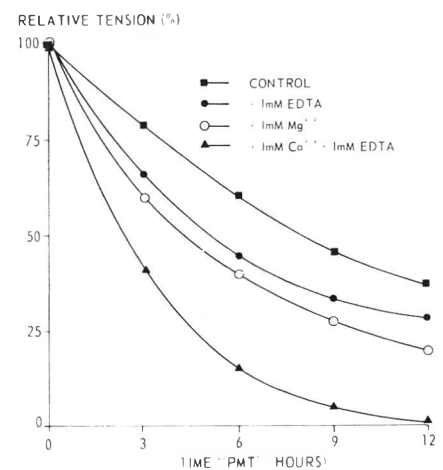


Fig. 4—Effect of 10<sup>-3</sup> M magnesium, EDTA and calcium-EDTA in extracellular buffer on tension release pattern for *P. major* muscle from chicken broilers. Each point is the average of five birds. (PMT = post-maximum tension)

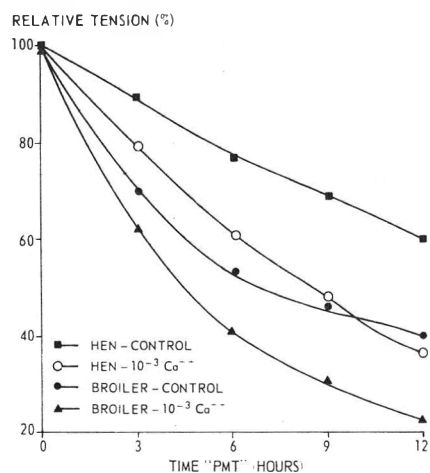


Fig. 5—Tension release patterns for *P. major* muscle of three groups of chicken broiler controls segregated on the basis of time required to reach maximum tension. Each point represents average of 10 birds (for Groups I and III) or 15 birds (Group II). (PMT = post-maximum tension).

lar time. These authors isolated a "calcium activated sarcoplasmic factor" from rabbit muscle and demonstrated its ability to effect complete Z-line removal from rabbit muscle myofibrils in the presence of at least 1 mM calcium. Although there is no direct evidence for the existence of such a factor in broiler muscle the marked increase in tension release by 1 mM calcium, but not by 0.1 mM, is suggestive of some type of concentration dependent calcium activation.

The effects of magnesium, EDTA and equimolar combinations of calcium-EDTA and magnesium-EDTA are shown in Table 4 and Figure 4. Time to maximum tension was not significantly affected by any of the treatments, a result which supports the suggestion of impermeability of the sarcolemma to extra-

cellular materials in early postmortem muscle. The amount of tension developed was significantly lower ( $P < 0.05$ ) than for controls except for the strips in buffer containing only magnesium.

The tension release findings were quite unexpected. EDTA, which has been reported to prevent tension release in porcine and rabbit muscle (Busch et al., 1972a, b) stimulated tension release in broiler breast muscle (Fig. 4). When 1 mM calcium or magnesium was added to buffer containing 1 mM EDTA an additive effect was observed on tension release. The data for magnesium-EDTA (not shown) were virtually the same as for calcium EDTA. The release pattern for strips in 1 mM magnesium was also similar to the calcium pattern shown in Figure 3. Because magnesium ions act as a

plasticizer in muscle it is possible that magnesium stimulates the dissociation of actomyosin resulting in an increased rate of tension decline. The rapid tension release observed in the presence of calcium and EDTA or magnesium and EDTA is more difficult to explain. It is possible that the uncharged chelate may pass through the sarcolemma and once inside the muscle, calcium or magnesium may be displaced by other ions such as zinc, lead or iron. The released calcium or magnesium could then stimulate tension release. Further experiments are underway to test this hypothesis.

Segregation of broiler controls on the basis of time to reach maximum tension

It was noted during the course of the

Table 4—Means and standard errors of time to maximum tension and tension developed for strips of broiler *P. major* muscle in phosphate buffer and buffer containing  $Mg^{++}$ , EDTA,  $Ca^{++}$  + EDTA and  $Mg^{++}$  + EDTA

Treatment	Time, hr	Tension, g/cm <sup>2</sup>
Control (n = 11)	5.74 ± 0.76	51.25 ± 2.36
10 <sup>-3</sup> M $Mg^{++}$ (n = 5)	6.48 ± 1.21 (6.44 ± 1.13) <sup>a</sup>	41.62 ± 4.81 (53.12 ± 3.79)
10 <sup>-3</sup> M EDTA (n = 5)	4.70 ± 0.96 (5.39 ± 1.27)	38.88 ± 1.17 <sup>b</sup> (50.12 ± 3.79)
10 <sup>-3</sup> M $Ca^{++}$ + 10 <sup>-3</sup> M EDTA (n = 5)	4.65 ± 0.96 (6.18 ± 1.48)	32.57 ± 3.73 <sup>b</sup> (49.22 ± 3.97)
10 <sup>-3</sup> M $Mg^{++}$ + 10 <sup>-3</sup> M EDTA (n = 3)	4.56 ± 0.95 (5.77 ± 0.89)	32.90 ± 0.95 <sup>b</sup> (56.14 ± 5.40)

<sup>a</sup> Values within parentheses are averages for control strips run simultaneously with the various treatment strips and used in the paired comparison analyses.

<sup>b</sup> Significantly different from control ( $P < 0.05$ )

Table 5—Means and standard deviations of time to maximum tension and tension developed for three groups of chicken broilers segregated on the time required to reach maximum tension

Group <sup>a</sup>	Time, min	Tension, g/cm <sup>2</sup>
I (n = 10)	147.2 <sup>b</sup> ± 30.3	47.74 ± 15.65
II (n = 15)	284.7 ± 40.3	46.09 ± 8.94
III (n = 10)	473.1 <sup>b</sup> ± 74.1	51.88 ± 12.32
Pooled (n = 35)	299.3 ± 134.7	48.50 ± 12.60

<sup>a</sup> Tension maximum for the three groups was observed between:  
Group I 0–3 hr postmortem  
Group II 3–6 hr postmortem  
Group III > 6 hr postmortem

<sup>b</sup> Significantly different from Group II ( $P < 0.01$ )

Table 6—Simple linear correlations of 2 hr tension release values with subsequent hourly values from three groups of control broilers and for the pooled data from the three groups.

Time, hr	Group I <sup>a</sup> (n = 10)	Group II (n = 15)	Group III (n = 10)	Pooled (n = 35)
4	0.965**	0.914**	0.866**	0.951**
6	0.889**	0.818**	0.767**	0.899**
8	0.873**	0.828**	0.689*	0.883**
10	0.845**	0.749**	0.506	0.841**
12	0.828**	0.695**	0.414	0.798**

<sup>a</sup> See footnote for Table 5 for Group designations

\*  $P < 0.05$

\*\*  $P < 0.01$

experiments on tension parameters that muscle strips run as controls varied considerably in the time to reach maximum tension and rate of tension release. There was a definite relationship between time to reach maximum tension and the proportion of tension released within 12 hr post-maximum tension. The data for control strips from 35 broilers were segregated into three groups and the tension parameters for these groups, as well as the pooled data, are presented in Table 5. The three groups did not differ in amount of tension developed, but did differ significantly ( $P < 0.01$ ) in time required to reach maximum tension. As shown in Figure 5, the birds in group 1 which reached maximum tension in less than 3 hr, released tension significantly more rapidly than did Group 2 or 3 birds ( $P < 0.01$ ). Groups 2 and 3 differed significantly ( $P < 0.05$ ) from each other in the amount of tension released during the first 5 hr post-maximum tension. The tension release pattern for Group 1 birds was almost identical to that observed when 1 mM calcium was added to the incubation buffer.

The differences in rate of tension release were apparent as early as 2 hr post-maximum tension, thus an attempt was made to determine if the percentage of tension released at this time was indicative of subsequent release rate. Thus the 2 hr release data for each of the three groups were correlated with the values for each of five subsequent times post-maximum tension. The Groups were analyzed individually and then pooled to establish a general relationship (Table 6). Significant linear correlations ( $P < 0.01$ ) were obtained in all instances except for 2 hr versus 10 and 12 hr for Group 3 birds. The pooled data show highly significant linear correlations ( $P < 0.01$ ) between percentage tension at 2 hr and at each of 4, 6, 8, 10 and 12 hr post-maximum tension.

#### Relation between 2 hr tension release, time to maximum tension and shear value

Analyses were performed to determine whether rate of tension release was significantly correlated to the time required to reach maximum tension. The data for strips for 120 birds showed a linear relationship between two hour tension release and time to maximum tension. With percent relative tension (% RT) at 2 hr

post maximum as the dependent variable and time to maximum tension as the independent variable the following equation for a regression line was obtained: % RT = 63.57 - 0.092 Time (hr);  $r^2 = 0.541$  ( $P < 0.01$ ).

The percentage of maximum tension released in 2 hr ranged from 0–80% and shear values of cooked muscles ranged from 2.46 to 18.43 lb (1.12 – 8.38 Kg). However, no significant relationship was found between the two variables suggesting that at least among individual birds the tension release observed is not indicative of tenderness at 24 hr postmortem.

The overall tension pattern for the pooled data was indicative of the tenderization process for broilers. Most tenderization in P. major muscle of broilers occurs within 12–24 hr postmortem (deFremery, 1966) and during this period 60% or more of the isometric tension was released. The tenderization process in broilers is much more rapid than in pork or beef muscle which show a slower rate of tension development and decline (Busch et al., 1972a). It may be concluded that relatively large differences in tension pattern are required to effect relatively small changes in tenderness.

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## COLD SHORTENING IN CHICKEN BROILER PECTORALIS MAJOR

### INTRODUCTION

TWO BASIC physical changes occur in pre-rigor postmortem muscle, a shortening or tendency to contract and a loss of extensibility. Factors which affect these changes and their relation to tenderness have been reviewed by Marsh (1972) and Newbold and Harris (1972).

Pre-rigor shortening varies greatly depending on temperature and not all muscles show the same extent of shortening. Locker and Hagyard (1963) defined a cold shortening phenomenon in ox neck muscle and since that report the phenomenon has been reported to occur in ovine (Cook and Langsworth, 1966), porcine (Galloway and Goll, 1967; Hendricks et al., 1971) and avian (Smith et al., 1969) muscles. Jungk and Marion (1970) found that turkey breast muscle did not cold shorten whereas thigh muscle did (Marion, 1971).

In most of the above instances, shortening was determined by measuring actual length changes. Since measurement of isometric tension is a measure of the tendency of the muscle to shorten and is much more sensitive than actual measurement of length changes (Busch et al., 1972) it follows that isometric tension measurement should be useful in the study of cold shortening. Wood and Richards (1974) used tension measurement to demonstrate such a tendency to shorten in chicken broiler breast muscle. The tendency to shorten was found to increase significantly as temperature decreased from 5 to 0°C.

The present study was initiated using isometric tension measurements to study the ability of muscle to develop tension and the level of energy rich phosphate subsequent to cold-induced tension development.

### EXPERIMENTAL

#### Experiment 1

Broilers, 8–12 wk of age were used in this study. Slaughter and preparation for tension measurement were carried out as previously described (Wood and Richards, 1974). Six muscle strips carefully cut to 5 cm in length were prepared from each bird and weighed. Cross sectional area of each strip was determined from a previously determined regression equation relating weight to area. Most strips

were between 0.15 and 0.25 cm<sup>2</sup>. Two strips were run in buffer at 22–25°C (23°C will be used henceforth in this paper to denote this temperature range). Four strips were attached to isometric transducers in a 2°C cold room and precooled buffer at 2°C was added to the incubation chambers. In total 36 strips, from 9 different birds, were run in buffer at 2°C. Twelve of these strips were removed from the cold room at each of 12, 24 and 36 hours post-maximum tension. The time to maximum tension was determined from strips run at 23°C. Upon removal from the cold room the 2°C buffer was exchanged for buffer at 23°C, the strips attached to transducers at 23°C and tension development followed. Another series of experiments was conducted in which 24 strips, from 6 birds, were attached to transducers at 23°C; 2°C buffer was added to 12 strips and 23°C buffer added to the other 12. Immediately after cold shortening had occurred in the 2°C buffer, it was replaced with buffer at 23°C and subsequent changes in tension monitored. A total of 15 birds was used so that each of the treatments contained data for at least 12 strips of muscle from at least 6 different birds (Table 1).

#### Experiment 2

Six broilers were used in this study. Birds were exsanguinated and a sample of breast muscle was immediately frozen in liquid nitrogen. Twelve strips of muscle of the size used for tension measurements, were prepared from each bird. Eight strips were placed in phosphate buffer (as used in tension studies) at 2°C and 4 placed in buffer at 23°C. After 3 min, 4 strips were removed from the cold buffer, dried

quickly on a paper towel, and frozen in liquid nitrogen. The remaining 8 strips were removed from the respective buffers at 7 min and similarly frozen. All frozen samples were stored under liquid nitrogen prior to analysis. The frozen samples were powdered by the method of Borchert and Briskey (1965) as modified by Vanderstoep and Richards (1974). Adenosine triphosphate (ATP), creatine phosphate and hexose monophosphate analyses were performed on the powdered samples according to the method of Lamprecht and Stein (1963).

### RESULTS & DISCUSSION

THE EFFECT of the initial cold shortening on the subsequent ability of muscle strips to develop tension is shown in Table 1. Muscle strips from the 15 broilers averaged 30.94g of tension within 3 min at 2°C. The tension developed through this cold shortening was released almost as rapidly as it developed and in most cases strips had returned to the original starting point within 15 min of attachment to the transducers.

When strips were allowed to shorten at 2°C, then brought to 23°C by changing buffer, considerable tension was developed subsequently during the course of a normal tension pattern ("0" time treatment). Compared to control strips, the time to reach maximum tension was about 70 min shorter and the amount of tension developed, about one-third lower.

Table 1—Means and standard errors of tension parameters of broiler P. major muscle and number of strips and birds for the various post-slaughter temperature treatments

Treatment	No. of Strips <sup>c</sup>	No. of Birds	Tension, g/cm <sup>2</sup>	Time, min
Control, 23°C	30	15	61.31 ± 2.7	331.67 ± 23.5
Cold shorten, 2°C	48	15	30.94 ± 5.9	2.93 ± 0.3
"0" time <sup>a</sup>	12	6	43.57 ± 8.1	261.83 ± 42.0
12 hr PMT <sup>b</sup>	12	9	12.62 ± 0.03	63.16 ± 11.8
24 hr PMT <sup>b</sup>	12	9	7.93 ± 0.03	102.67 ± 35.5
36 hr PMT <sup>b</sup>	12	9	1.77 ± 0.8	40.5 ± 10.3

<sup>a</sup> "0" time refers to strips placed in buffer at 2°C to observe the cold shortening effect then immediately subjected to buffer at 23°C to observe further tension development. Elapsed time from slaughter to placement in 23°C buffer averaged 10 min.

<sup>b</sup> Hours post maximum tension of exposure of muscle strips in buffer at 2°C prior to replacement in buffer at 23°C. The time to maximum tension was determined from corresponding control strips run at 23°C.

<sup>c</sup> For each treatment, no more than 2 strips per bird are represented except for the "Cold shorten, 2°C" treatment where 9 birds are represented by 4 strips each and 6 birds by 2 strips each.

<sup>1</sup> Present address: Agriculture Canada Research Station, Summerland, B.C.



Muscle strips held in buffer at 2°C for periods of 12, 24 or 36 hr did not show any tension development after the initial cold shortening. These strips also demonstrated a decreasing ability to develop isometric tension when raised to 23°C after the various storage times. At 36 hr post-maximum tension, the strips had essentially lost their ability to develop further tension when brought to 23°C. This suggests that glycolysis had continued in the strips at 2°C but the rate was probably too slow to effect a tension development, other than the initial cold shortening tension, at this temperature.

The mechanism of the cold shortening phenomenon is still somewhat obscure. Locker and Hagyard (1963) showed that cold shortening, in beef muscle, started within a few minutes of commencement of cooling and was almost complete within 1 hr. Smith et al. (1969) found that cold shortening was maximal in chicken breast muscle at 0°C and was essentially complete after 3 hr post-mortem. The variations between these studies and the present study reflect differences in size of muscle used and the sensitivity of isometric tension measurement versus measurement of length changes.

In order to better understand the nature of the cold shortening, a study was initiated to measure the effect of cold shortening on some of the energy-rich phosphate compounds in chicken muscle. The effect of cold shortening on ATP levels is of particular interest since shortening occurs only in the presence of ATP. The results of this study are shown in Table 2.

ATP levels were lowered somewhat by subjecting strips of muscle to buffer at 2°C for 3 or 7 min prior to freezing in liquid nitrogen. A lower ATP level was also evident in the muscle strips held in buffer at 23°C. Only the samples held in cold buffer for 7 min differed significantly ( $P < 0.05$ ) from samples frozen at death. However, these cold treated samples did not differ from strips held for 7

min in buffer at 23°C. This lack of a significant decrease in ATP level is in accord with the findings of Busch et al. (1967) who observed only a small change in ATP level during large tension development at 2°C. It is evident, however, that a significant amount of ATP need not be hydrolyzed to effect cold shortening as observed in chicken broiler breast muscle. Normal tetanic contraction in muscle is capable of developing 2–3 Kg tension/cm<sup>2</sup> muscle (Huxley, 1958). The 30.9g/cm<sup>2</sup> observed on cold shortening represents only about 1% of the potential tetanus, therefore, only minor changes in ATP level might be expected. The presence of adequate levels of ATP subsequent to shortening would account for the ability of the muscle to relax to rest level immediately after cold shortening has occurred.

Marsh (1966) postulated that cold shortening, like thaw rigor, resulted from inactivation of a relaxing factor by calcium release, this being the result of a salt "flux." Such a salt "flux" could be explained in terms of the relative rates of diffusive and chemical processes since the temperature coefficient of diffusion is considerably lower than that of chemical reaction.

Applying this postulation to the cold shortening phenomenon observed in broiler muscle, it is possible to suggest the following sequence of events in the muscle when subjected to cold buffer at 2°C. A salt "flux" of calcium ions, released from the sarcoplasmic reticulum (SR), would stimulate myofibrillar adenosine triphosphatase (myosin) to split ATP thus effecting shortening or contraction. The extent of the shortening would be proportional to the concentration of the salt "flux." This "flux" apparently varies substantially between 5 and 0°C since tension increases significantly as temperature approaches 0°C (Wood and Richards, 1974). The salt "flux" effect may be a temporary phenomenon in broiler muscle resulting from the "cold shock" and it would appear that after temporarily

losing the ability to sequester calcium, the SR again becomes operable, removing calcium from the myofibrils. Since considerable ATP is still present, to act as a plasticizer, the muscle relaxes to its original starting tension. If held at or near 0°C, the strips maintain a slow rate of ATP breakdown which is not rapid enough to effect further tension development. After 36 hr, at or near 0°C, sufficient ATP catabolism has occurred, within the muscle strips, to eliminate any further ability to develop tension even at 23°C.

The observed decrease in hexose monophosphate levels may result from an increased phosphorylation of fructose-6-phosphate to fructose-1-6-diphosphate. However, further study is required to verify this finding.

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Table 2—Means and standard errors of the ATP, HMP and CP<sup>a</sup> content of broiler P. major muscle subjected to various temperature treatments ( $\mu$  moles/g tissue)

Treatment	HMP	ATP	CP
Control	6.95 ± 0.68	9.23 ± 0.64	2.02 ± 0.40
3 min at 2°C	4.48 ± 0.56**	8.40 ± 0.49	2.23 ± 0.24
7 min at 2°C	4.17 ± 0.52**	8.06 ± 0.51*	2.52 ± 0.25
7 min at 25°C	5.35 ± 0.36	8.56 ± 0.55	2.44 ± 0.21

<sup>a</sup> Abbreviations: HMP, hexose monophosphate; ATP, adenosine triphosphate; CP, creatine phosphate

\* Significantly different from control ( $P \leq 0.05$ )

\*\*  $P \leq 0.01$

## CHANGES IN TENDERNESS OF BEEF LONGISSIMUS DORSI AS RELATED TO MUSCLE COLOR AND pH

### INTRODUCTION

TENDERNESS of muscle has been shown to be pH dependent in beef (Martin et al., 1971), rabbits (Miles and Lawrie, 1970) and sheep (Bouton et al., 1971). A positive association between color intensity and pH has been demonstrated and a procedure described for utilizing pH of the longissimus dorsi to identify potential dark cutting beef carcasses (Munns and Burrell, 1966).

Postmortem time trends of muscle pH are influenced in some degree by the muscle chosen (Lawrie et al., 1959; Martin and Fredeen, 1973) and there is research evidence that ultimate pH (13–16 days after slaughter) is slightly higher than at 48 hr (Wierbicki et al., 1956). The purpose of the research documented in this paper was to investigate the utility of initial pH (immediately after slaughter) as a predictor of ultimate pH, meat color and tenderness of beef muscle, and to study

the influence of sex and source of the animals on these traits.

### MATERIALS & METHODS

THIS STUDY involved carcasses of 574 animals of three sexes (168 steers, 147 heifers and 259 bulls). These were obtained from three sources, commercial feed lots in central Alberta and the C.D.A. Research Stations at Lacombe and Lethbridge. All were transported by truck to the same abattoir and slaughtered within 1 hr after delivery. Total transportation time for the Lethbridge animals, 66 steers and 197 bulls, was approximately 7 hr. The remaining animals were in transport for less than one hour. Age at slaughter ranged from 12–16 months. Additional details of the pre-slaughter history of these animals have been published by Fredeen et al. (1971).

The initial pH reading was taken approximately 20 min after slaughter by direct insertion of a probe-type electrode into the trapezius cervicales. A second pH reading was taken in this same muscle 22–26 hr postmortem. At this time pH was also recorded for the longissi-

mus dorsi at the 11–12 rib interface with additional pH readings taken on this muscle at 48 hr, 72 hr, 6 days and 13 days postmortem.

The pH readings were taken with an ILI (Instrumentation Laboratory Inc.) Portomatic pH meter model no. 175 fitted with a spike tip electrode specifically adapted for meat samples. Calibration was according to manufacturer's instructions. The meat temperatures for all except the initial pH ranged from 1–2°C.

Color of the longissimus dorsi cross section at the 11–12 rib was rated subjectively at 24 hr postmortem using a scale of 1 (light) to 3 (dark). Color of the same muscle was also measured objectively by a photo electric brightness meter manufactured by Ernst Schütt Laboratories, Göttingen, Germany. This meter operated through a range of 0 to 100 with higher values associated with increasing darkness of the meat surface.

As each carcass was processed at 24 hr postmortem the excised L. dorsi from the left rib cut (4th to 11th rib) and the untrimmed short loin from the right side (13th rib to 5th lumbar vertebra) were taken to the laboratory for storage at 2°C (± 1°) and 94% (± 6%) relative

Table 1—Means and standard errors for pH, shear values and meat color for beef carcasses of 6 sex-source sub-groups

	Steers				Heifers				Bulls			
	Comm		Leth		Comm		Lac		Lac		Leth	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
No. of carcasses	102		66		100		47		85		174	
Initial pH (0 hrs)	7.03	0.02	6.98	0.02	6.89	0.02	7.19	0.02	7.01	0.02	7.05	0.02
pH l. dorsi 48 hrs	5.56	0.01	5.68	0.01	5.57	0.02	5.68	0.02	5.64	0.02	6.09	0.03
Final pH (13 days)	5.67	0.02	5.66	0.02	5.60	0.02	5.73	0.02	5.76	0.02	6.12	0.03
Post mortem change in pH												
(Initial vs. 24 hr t. cerv.)	1.21	0.03	1.05	0.04	0.99	0.03	0.97	0.04	1.06	0.03	1.23	0.03
(Initial vs. 24 hr l. dorsi)	1.42	0.02	1.26	0.03	1.15	0.04	0.91	0.04	1.26	0.03	0.96	0.04
(24 vs. 48 hr l. dorsi)	0.05	0.02	0.04	0.03	0.17	0.02	0.60	0.03	0.11	0.02	-0.03	0.02
Subjective color score	1.03	0.02	1.11	0.04	1.04	0.02	1.00	0.00	1.05	0.03	1.86	0.06
Color brightness	54.3	1.0	62.8	1.0	61.5	1.0	50.6	1.6	57.6	1.3	73.0	0.9
Shear values												
l. dorsi (proximal) 24 hr	71.4	1.5	60.9	1.6	68.6	1.5	57.5	1.6	79.4	1.6	55.8	1.6
change 24–48 hr	7.3	0.8	5.2	0.09	7.7	1.1	2.9	1.3	8.1	1.0	3.4	0.8
change 48–72 hr	5.5	0.7	5.9	0.07	0.1	1.0	3.2	1.2	4.9	0.9	2.6	0.7
l. dorsi (distal) 48 hr	75.6	1.5	69.0	1.8	81.7	1.6	76.9	2.5	82.7	1.4	70.1	1.6
change 48–72 hr	-1.0	1.2	-5.6	1.5	-0.7	1.2	-0.9	1.6	-2.8	1.2	1.5	0.9
change 48 hr–6 day	12.0	1.2	18.5	1.3	10.6	1.1	11.4	1.3	6.6	1.1	8.6	0.8
change 6 day–13 day	5.4	1.0	4.0	1.2	10.1	1.2	0.9	1.3	10.6	1.0	6.4	0.8
Total change in tenderness as a % of initial value												
% change in rib to 72 hr	17.9	1.3	18.2	2.3	9.1	2.0	10.6	2.4	16.3	1.7	7.8	1.7
% change in loin to 13 day	20.2	1.4	24.5	2.1	23.3	1.5	14.8	2.3	17.4	1.6	21.5	1.4

humidity pending subsequent sampling. Serial samples of these two sections were taken for tenderness evaluation at 24, 48 and 72 hr for the rib section and 48 hr, 72 hr, 6 days and 13 days postmortem for the loin section. Each sample was a cross sectional slice 3.2 cm thick with such trimming between sampling periods as required to provide a "fresh" surface of each slice. These slices were cooked according to a standardized procedure in a microwave oven, sealed in plastic and refrigerated at 1°C for 18–24 hr prior to coring for tenderness evaluation. Three cores, each 2 cm in diameter, were sheared in a Warner-Bratzler apparatus with the shear force electronically recorded on a scale 0–100.

Additional details of these procedures have been published by Martin et al. (1970; 1971).

Standard statistical analyses of variance and covariance were applied to the data. For comparison of mean values between sexes and locations (Table 1) a difference greater than 2.8 times the larger standard error is expected to be statistically significant ( $P \leq 0.05$ ). A more precise test of significance would be given by the relationship

$$t' = (w_1 t_1 + w_2 t_2) / w_1 w_2$$

where  $w_1 = SE_1^2$ ,  $w_2 = SE_2^2$  and  $t_1$  and  $t_2$  are the  $t$  values indicated by the degree of freedom involved with  $SE_1$  and  $SE_2$  respectively.

RESULTS & DISCUSSION

INITIAL pH recorded 20 min after slaughter averaged almost exactly 7.0 for bulls, steers and heifers. None of the 574 carcasses had a pH less than 6.2 and 93% were within the pH range of 6.7–7.3. Source of the bulls and steers had no influence on this pH reading (Table 1) but the two groups of heifers differed by 0.30 ( $P < 0.01$ ).

Lethbridge bulls differed singularly from commercial steers and commercial heifers in their frequency distribution for 24 hr pH of the longissimus dorsi (Fig. 1). They ranged from 5.3–7.0 with 49.2% in excess of 6.0 whereas none of the steers and only 8% of the heifers had a pH greater than 6.0.

The minimum pH for all sex-source sub-groups was recorded at 48 hr post-mortem but the change from 24 to 48 hr was statistically nonsignificant except for heifers. This sex showed a source difference with the commercial heifers showing a greater pH change from 0–24 hr (1.15 vs. 0.91) and less from 24–48 hr (0.17 vs. 0.60) than the Lacombe heifers. Final pH (13 days) was similar for steers, heifers

and Lacombe bulls but the Lethbridge bulls maintained an average pH in excess of 6.0 through this period. Except for the Lethbridge steers, all groups showed a slight rise in pH of the longissimus dorsi over the period from 48 hr to 13 days postmortem.

From comparison of the results obtained for Lacombe vs. Lethbridge bulls it seems clear that duration of pre-slaughter stress associated with transport (1 hr vs. 7 hr) has an important influence on post-mortem changes in pH and tenderness. Differences in transport time and/or other factors associated with source had only a small effect on steers (i.e., Lethbridge vs. commercial steers) but appeared to have a pronounced effect on bulls (i.e., steers vs. bulls from Lethbridge).

Sex and source differences were also evident in meat color and shear values (Table 1). The highest color scores, both subjective and objective and the lowest initial shear values were recorded for the Lethbridge bulls. Lacombe bulls, with a significantly lower color rating ( $P < 0.01$ ) had substantially higher shear values ( $P < 0.01$ ). The same pattern of association between average color brightness score and initial shear values was observed with the steers where the group with the lowest average color rating (commercial steers) had significantly higher shear values. For heifers, this association was reversed (Table 1).

Meat color and tenderness relationships with 24-hr pH are illustrated graphically in Figure 2 for Lethbridge bulls, commercial steers and commercial heifers. Over the pH range shared in common by these three sex-source groups (i.e., below 6.0) there was no evidence of correlation between 24 hr pH and either color or tenderness. However, definite trends were established as pH increased above 6.0 and bulls, which had this pH range almost exclusively to themselves, produced rather different results than steers and heifers.

Because of the sex differences in pH range illustrated by Figure 2 the pooling of data for correlation studies was considered statistically unsound. Accordingly all correlations were estimated within sex and source and those presented in Tables 2 and 3 relate only to three sex-source groups identified in Figure 2, namely commercial steers, commercial heifers and Lethbridge bulls.

The pH readings almost invariably dropped during the first 24 hr post-mortem whether the second measurement was taken in the trapezius cervicales or the longissimus dorsi and the correlations between initial pH value (0 hr) and pH change to 24 hr were high and positive. However, the correlations between the absolute values for initial and 24 or 48 hr pH were generally small and nonsignifi-

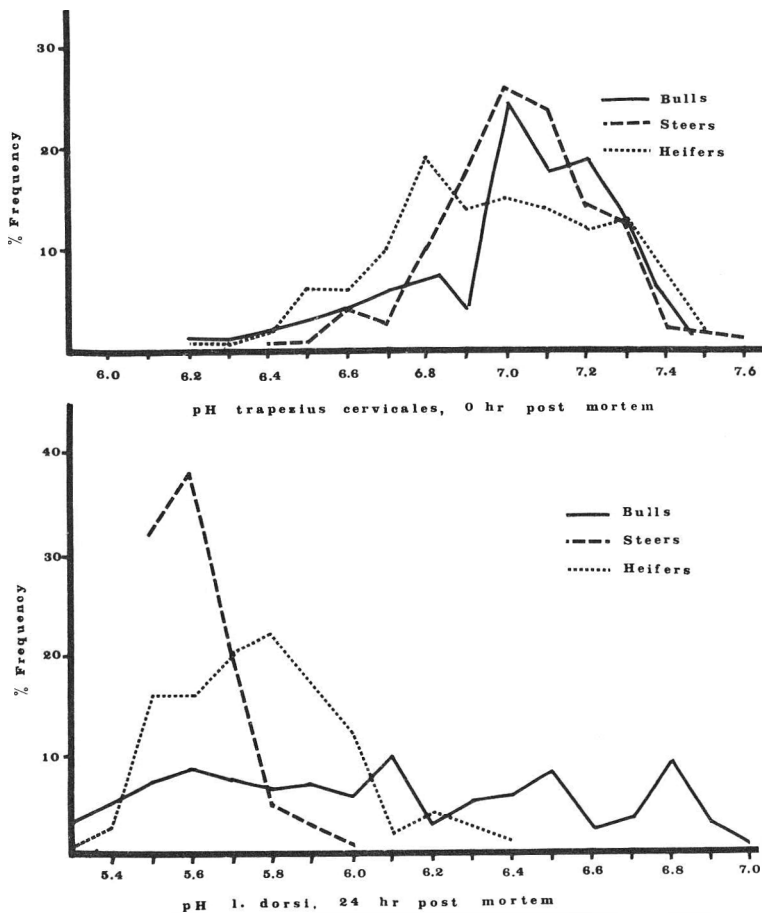


Fig. 1—Frequency distribution of pH at 0 hr (trapezius cervicales) and 24 hr (l. dorsi) for bulls, steers and heifers.

cant, indicating that the variance observed in one reading accounted for less than 10% of the variance in a subsequent reading. The only exceptions were the correlations between the 24 and 48 hr pH readings of the longissimus dorsi for bulls (0.82) and heifers (0.46). There were sex differences in the sign of statistically significant ( $P < 0.01$ ) correlations but, because of the low coefficients of determination involved (i.e.,  $r^2$  values) these sex differences would appear to be of limited importance (Table 2).

Initial tenderness (shear value) of the rib and loin sections of the longissimus dorsi were not predictable from either of the two pH readings taken on the trapezius cervicales and only for bulls were the pH readings on the longissimus dorsi itself meaningfully correlated with tenderness. For this sub-class, high pH readings at 24 or 48 hr were associated with low shear values (Table 3). However, none of the pH readings or changes in pH were useful in predicting the magnitude of change in tenderness which occurred during the aging periods.

Although initial pH as here recorded on the trapezius cervicales was of no value for predicting either the rate or extent of postmortem pH or tenderization of the longissimus dorsi, the fact that muscles differ in their postmortem pH gradients (Lawrie et al., 1959; Martin and Fredeen, 1973) might suggest that use of a different muscle and/or time could alter this conclusion. It has also been suggested (Khan and Lentz, 1973) that pH measurements of the semimembranosus and adductor muscles are more definitive predictors than those taken on the trapezius cervicales. To determine whether the results documented in the present report were specific to the muscle chosen for initial pH, a separate study was conducted on 115 carcasses of bulls, steers and heifers with initial pH measurements taken on all three muscles 45 min postmortem. Correlations of these three initial pH values with 24-hr postmortem pH, pH change and tenderness of the longissimus dorsi did not differ statistically and supported the conclusions reached in the present study.

As shown by the averages in Table 1, subjective color scores were virtually invariant for carcasses from steers and heifers (i.e., a score of 1 for most carcasses) and scores of 3 were assigned only to dark cutting bulls. As a result, correlations of subjective vs. objective color ratings were significantly higher ( $P \leq 0.001$ ) for the bulls (0.70) than for steers (0.22) and heifers (0.38). The correlations of color with pH (Table 2) and shear values (Table 3) for Lethbridge bulls and commercial steers and heifers include both methods of color determination but discussion will center on those involving objective color measurements.

For bulls, the correlations of objective color with 24 and 48 hr postmortem pH of the longissimus dorsi were significantly higher ( $P \leq 0.001$ ) than for the other two sexes (Table 2). The negative correlation for bulls between color and pH change from 0 hr (t. cervicales) to 24 hr (l. dorsi), also significantly higher ( $P \leq 0.001$ ) than for steers and heifers, demonstrated that muscle retaining a high postmortem pH tended to be dark in color.

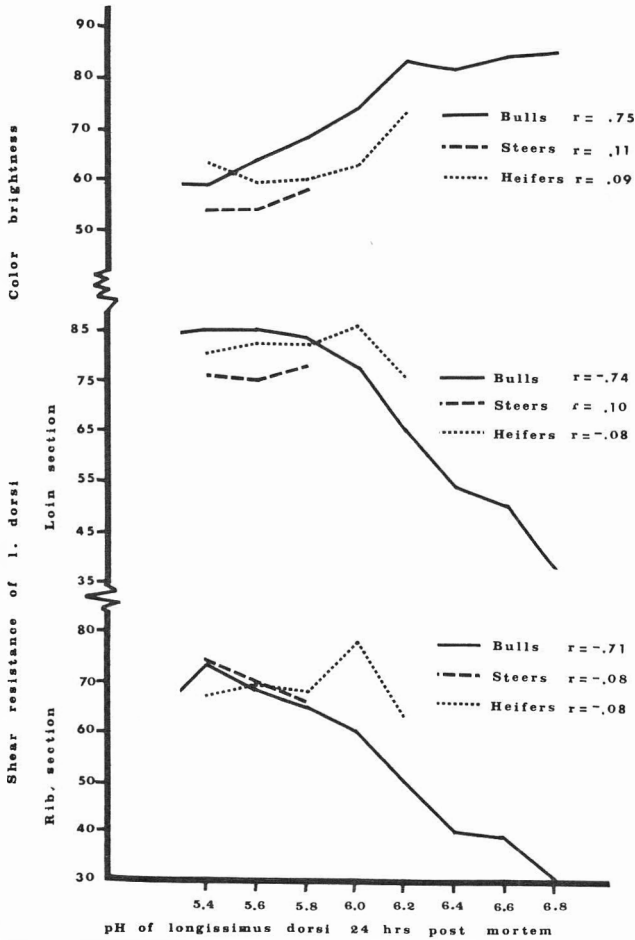


Fig. 2—Relationship of color brightness and two measures of shear resistance (tenderness) with 24 hr postmortem pH of the longissimus dorsi.

Table 2—Correlations among pH readings, postmortem changes in pH and color of longissimus dorsi for bulls, steers and heifers

		pH		pH of	
		t. cervicales		l. dorsi	
		0 hr	24 hr	24 hr	Color of l. dorsi
				Subjective	Objective
pH t. cervicales:					
0 hr	Bulls			.27	.23
	Steers			.14	-.01
	Heifers			.12	.45
24 hr	Bulls	-.02		.05	.10
	Steers	-.10		.08	.11
	Heifers	-.09		-.28	-.14
pH l. dorsi:					
24 hr	Bulls	.18	.14	.71	.75
	Steers	.13	-.07	.47	.11
	Heifers	-.14	.29	.48	.09
48 hr	Bulls	.28	.09	.82	.73
	Steers	-.26	.26	.02	-.03
	Heifers	-.01	-.29	.46	.24
pH change from 0 hr for t. cervicales to:					
24 hr t. cerv.	Bulls	.79		.17	.08
	Steers	.72		.04	-.08
	Heifers	.83		.25	.43
24 hr l. dorsi	Bulls	.35	-.22	-.55	-.60
	Steers	.83	-.06	-.14	-.07
	Heifers	.79	-.24	-.21	.26
Absolute magnitude of correlations required for significance					
		P < 0.05		P < 0.01	
	Bulls	0.15		0.19	
	Steers	0.20		0.25	
	Heifers	0.20		0.25	

Table 3—Correlations of pH readings and muscle color ratings with estimates of tenderness (shear force) for bulls, steers and heifers

	pH of t. cervicales		pH of l. dorsi		Color of l. dorsi	
	0 hr	24 hr	24 hr	48 hr	Subjective	Objective
Shear force, rib section of l. dorsi at 24 hr						
Bulls	-.23	-.09	-.71	-.68	-.61	-.56
Steers	.06	-.05	-.08	-.09	-.02	.33
Heifers	.08	.06	-.08	-.20	-.26	.06
Shear force, short loin section of l. dorsi at 48 hr						
Bulls	-.15	-.11	-.74	-.74	-.55	-.55
Steers	-.02	-.16	.10	.07	.17	.29
Heifers	-.01	.11	-.08	-.25	-.28	.07
% Change in shear force for rib section 24 to 72 hr						
Bulls	.00	-.02	-.17	-.18	-.08	-.10
Steers	.12	-.12	.22	.07	.11	.26
Heifers	.16	-.14	-.12	-.12	.01	.09
% Change in shear force for short loin section 48 hr to 13 days						
Bulls	-.08	-.06	-.22	-.28	-.27	-.24
Steers	-.08	.18	-.01	.03	-.08	.13
Heifers	.14	.00	-.09	-.24	-.13	-.09
Absolute magnitude of correlations required for significance:						
			P < 0.05	P < 0.01		
	Bulls		0.15	0.19		
	Steers		0.20	0.25		
	Heifers		0.20	0.25		

For bulls there was an inverse relationship between objective color and shear value with increasing (darker) color intensity associated with decreasing (more tender) shear values (Table 3). However, sex differences for these correlations were highly significant ( $P \leq 0.001$ ) with opposite signs for bulls and steers. This latter observation is interpreted as evidence that dark color is a meaningful indicator of tenderness only at the upper extreme of the color range recorded in this study.

For the Lethbridge bull carcasses the importance of high 24-hr pH values of the longissimus dorsi in determining the nature of the pH-meat color and/or pH-shear force relationships is portrayed by Figure 3. 48% of these carcasses had a pH of 6.1 or greater. Of these, 79% were below average in shear force (53.5) at 13 days postmortem (i.e., more tender than average) and 75% had color scores of 80 or above (i.e., darker than average). Viewed in the context of meat color (Fig. 4), 85% of the carcasses with color scores of 80 or above were below average in shear force and 84% had a pH greater than 6.0. These observations suggest that

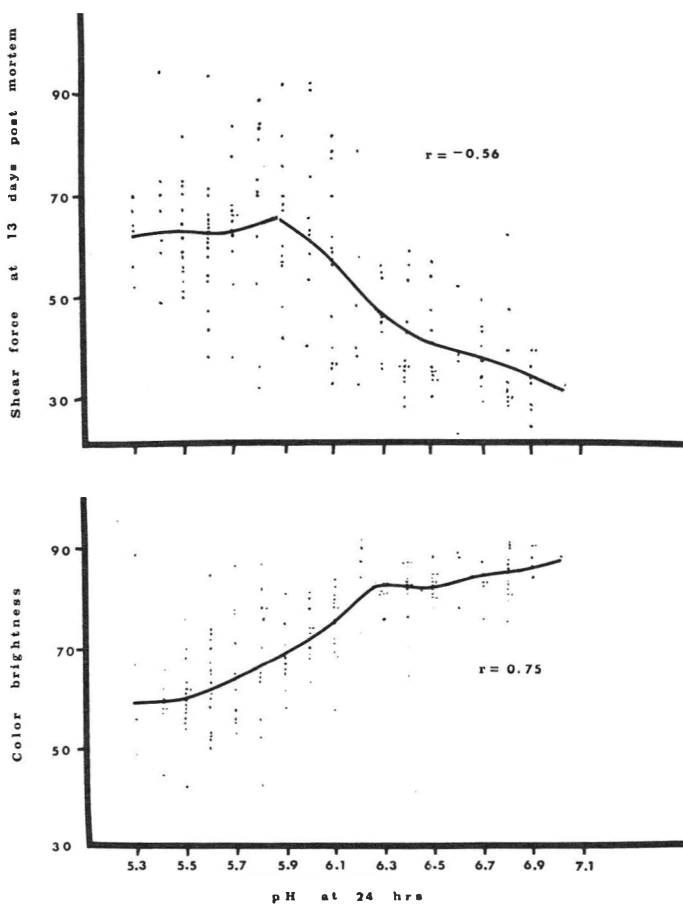


Fig. 3—Scatter diagrams of relationships between pH of longissimus dorsi at 24 hr postmortem and color brightness and shear values (bulls only).

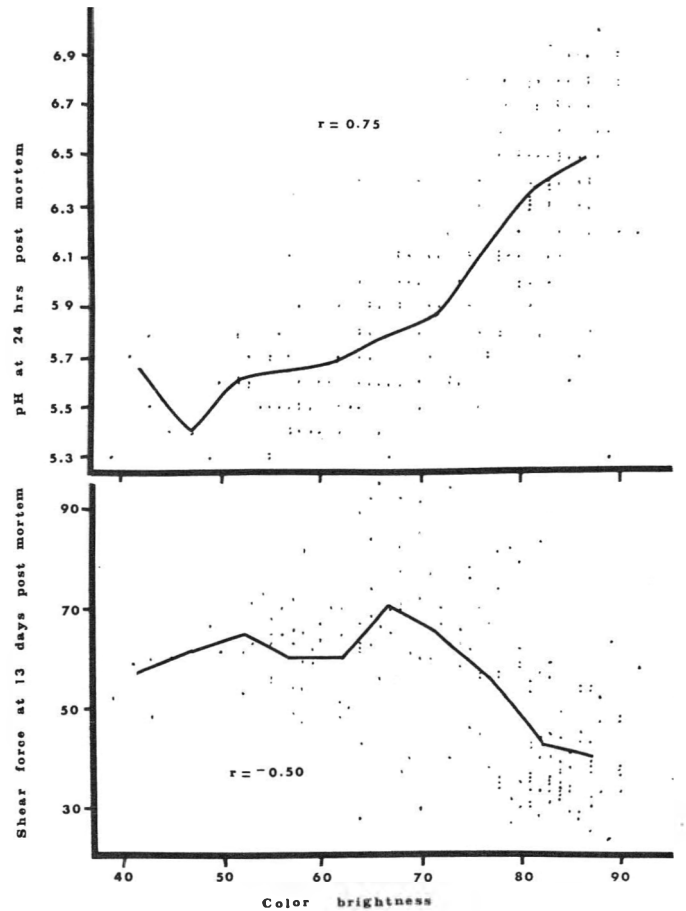


Fig. 4—Scatter diagram of relationships between color brightness of the longissimus dorsi at 24 hr postmortem and pH of the same muscle at this time and shear value at 13 days postmortem.

meat color may merit consideration as a technique for predicting potential tenderness of bull carcasses. However, this study provides no evidence that the same criterion of color would be appropriate to carcasses from steers or heifers. Further, dark cutting beef is considered undesirable by the meat trade and its potential for superior tenderness is unlikely to modify this view. It is also possible that beef exhibiting extreme tenderness will not in fact meet with high consumer acceptance.

Kahn and Lentz (1973), reporting results obtained from a study of 10 carcasses from each extreme of the immediate post-slaughter pH range, recommended that initial pH be used as a means for segregating carcasses with respect to rate of pH decline and after-aging tenderness. Results from the present study do not support this recommendation but there is good evidence that 24-hr pH or

meat color may have utility for this purpose. The fact that these results relate to a large unselected population of carcasses provides confidence in their general applicability to industrial situations.

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## THE AROMA OF CANNED BEEF: APPLICATION OF REGRESSION MODELS RELATING SENSORY AND CHEMICAL DATA

### INTRODUCTION

THE PURPOSE of determining relations between instrumental and sensory data may be to supplement or complement the panel service by an instrumental technique in quality control and in process and product development. This subject has previously been investigated by e.g., Biggers et al. (1969), Powers (1968), Powers and Keith (1968), Powers et al. (1971), Bednarczyk and Kramer (1971), Biswas and Biswas (1971), Biswas et al. (1971) and Vuataz and Reymond (1971). The literature concerning this subject was reviewed by Persson et al. (1973b). Besides the work quoted there, two interesting papers have recently been published. Fore et al. (1972) applied linear regression analysis on gas chromatographic data and sensory flavor scores from stored peanut butter, and found very high correlations between flavor scores and e.g., the ratio between 2-methyl propanal and hexanal. Dravnieks et al. (1973) have successfully applied stepwise discriminant analysis to classify corn odor by gas chromatographic technique. This work is of special value since the generality of the relations, calculated from a set of reference samples, were tested on an independent set of "unknown" samples.

In the present investigation the correlation technique, described by von Sydow (1971), Persson and von Sydow (1972) and Persson et al. (1974) has been applied. The latter work is dealing with the aroma of canned beef and this paper is a direct continuation. New models have been developed, which are more interpretable and which give an improved fitting to data. The purpose of the present investigation is to determine general relations between instrumental and sensory aroma data from a reference material consisting of a large number of different types of beef samples analyzed during several years. The relations obtained in this way have been tested on independent "unknown" samples. The relations obtained were classified into ad hoc, predictive and, eventually, causative ones. The meaning of this will be discussed in con-

nection with quality control and product and process development.

### EXPERIMENTAL

#### Materials and processing

Minced beef from top side round steaks (Biceps femoris and Vastus labialis) was added with fat and starch in four formulations. Details about the formulations are given elsewhere (Persson and von Sydow, 1973, 1974; Persson et al., 1973a). 35 samples were used as "reference samples" (see above) which were treated in various ways: variation in formulations and heating times (Persson and von Sydow, 1973), HTST-sterilization, sterilization in different packaging materials (cans and flexible pouches), "aseptic canning" (direct steam inlet) (Persson and von Sydow, 1974). As "unknown samples" specimens were used from "aseptic canning" (over-pressure by nitrogen) and from storage experiments (Persson and von Sydow, 1974).

#### GC-analysis

Data and details about the analytical procedures are given in Persson and von Sydow (1973; 1974). The quantitative determination of the volatiles in the headspace gas of canned beef samples was performed by using a sampling technique making it possible to analyze large volumes. Both a flame ionization detector (FID) and a sulfur specific flame photometric detector (S-FPD) were used. The components

were identified by analyzing the headspace gas of a low temperature distillate on a combined gas chromatograph-mass spectrometer. 95 compounds were identified and 23 of those were chosen (Table 1) to be used in the correlation analyses (Persson et al., 1973b).

The investigations took place during a period of 2 years. In order to control whether the accuracy and the sensitivity of the gas chromatographic system were constant during this time, the samples from all the sub-experiments were stored at  $-90^{\circ}\text{C}$  and a few samples from each sub-experiment were analyzed at the end of the whole experimental series. Some discrepancy was obtained for hydrogen sulfide in the 12 samples described by Persson and von Sydow (1973). This was corrected for in the regression calculations.

#### Sensory evaluation

For details in procedure, see Persson et al. (1973a). The samples were assessed using the odor quality technique: a panel was trained to recognize different odor notes in a food item and to estimate the intensities of these on a 10-point scale. Originally, a list of 28 odor notes was used to describe the sensory aroma properties of canned beef, but this was later reduced to 11 terms. Examples of odor qualities used were: "retort flavor," "sulfurous," "burnt," "meaty (cooked)," "sickly," "sharp," "pungent" and "acid, sour." The validity of the

Table 1—The 23 chemical compounds applied to the models<sup>a</sup>

Peak no.	Compound	Peak no.	Compound
4	Ethanal	S2	Hydrogen sulfide
12	Butanal	S4	Methyl mercaptan
22	Pentanal	S5	Ethyl mercaptan
32	Hexanal	S6	Dimethyl sulfide
47	Heptanal	S7	Methyl ethyl sulfide
9	2-Methyl propanal	S8	Ethylene sulfide
18	2-Methyl butanal	S10	Thiophene
17	3-Methyl butanal	S11	Dimethyl disulfide
		S12	2-Methyl thiophene
6a	Furan	S13	3,5-Dimethyl-1,2,4-trithiolane
26	2-Ethyl furan		
63	2-Pentyl furan		
13a	2,3-Butandione		
23	2,3-Pentandione		

<sup>a</sup> Peak numbers: 4, 12, 22, etc., and S2, S4, S5, etc., refer to retention orders on a SF 96 column and a Chromosorb G AW column, respectively.

<sup>1</sup> Present address: Nordreco AB, S-267 00 Bjuv, Sweden

odor quality assessment technique was tested using a specially designed statistical procedure, showing that most of the odor notes could be measured by unidimensional scales. A homogeneous group of panelists showing both high consistency and discriminability was selected. In addition, preference tests were performed. Data are given in Persson et al. (1973a) and in Persson and von Sydow (1974).

#### Psychophysical models used

To determine relations between the intensity values (R) for an odor quality and the corresponding gas chromatographic data (S) three basic models were used:

- (A)  $R = a \cdot \log S + b$   
 (B)  $R = c \cdot S^n + d$   
 (C)  $R = c \cdot S + d$

S was considered as a composed argument of different combinations of chemical compounds [ $S = f(x)$ ], including up to four compounds at a time.

The validity of the models was tested by means of linear regression analysis. To carry out the analyses a computer program was con-

**Table 2—Additional  $S = f(x)$ , where  $x$  is the concentration of different aroma compounds and  $S$  is calculated stimulus values used in the psychophysical models.  $w_i, w_j, \dots$  are weighting factors ( $\sum w_i = 1$ )**

(15)	$S = \sqrt[3]{x_i \cdot x_j \cdot x_k}$
(16)	$S = \sqrt[4]{x_i \cdot x_j \cdot x_k \cdot x_l}$
(2a)	$S = w_i \cdot x_i + w_j \cdot x_j$
(3a)	$S = w_i \cdot x_i + w_j \cdot x_j + w_k \cdot x_k$
(4a)	$S = w_i \cdot x_i + w_j \cdot x_j + w_k \cdot x_k + w_l \cdot x_l$

structed. It was possible to select and test any of the three basic models and any of the combinations of the compounds, i.e., any of  $S = f(x)$ .  $S = f(x)$  was calculated for all possible combinations of compounds. The program ran through all odor qualities for every basic model and for every combination of compounds. The models were ranked according to goodness of

fit. For details about models and testing procedures, see Persson et al. (1973b).

In this part of the investigation the list over tested arguments for S had been increased with five new peak combinations (Table 2). Combinations (15) and (16) (geometric means) were directly included in the original program. Additional changes in this program were the possibilities to rank the models either after the chi-square measure or after the correlation coefficient. For every model the mean difference between observed and calculated (from the regression line) R-data were determined: M.D. (= mean difference) =  $\frac{\sum (R_{obs} - R_{cal})}{N}$  and also the corresponding maximal difference was calculated: Max.D. (= max. difference) =  $\max (R_{obs} - R_{cal})$ ,  $1 \leq i \leq N$ , where N is the number of observational points. These types of differences have also been applied to evaluate the accuracy of predicted sensory data in "unknown" samples.

The arguments (2a), (3a) and (4a) had weighting factors ( $w_i$ ) in accordance with the suggestions made by Persson et al. (1973b). The  $w_i$ -values were calculated according to a maximum likelihood (ML) estimation procedure in

**Table 3—Examples of best outcomes for the odor quality "retort flavor" when the models described have been applied to the 35 reference samples<sup>a</sup>**

Without weighting factors				With weighting factors			
No.	Model	$r^b$	M.D. <sup>c</sup> when predicting unknowns	No.	Model	$r^b$	M.D. <sup>c</sup> when predicting unknowns
1	$R = c \cdot (x_{S11} + x_{S10}) + d$	0.62	0.43	1w	$R = c \cdot (0.32 \cdot x_{S11} + 0.68 \cdot x_{S10}) + d$	0.71	0.39
2	$R = c \cdot (x_{S7} + x_{S11} + x_{S10} + x_{S12}) + d$	0.68	0.39	2w	$R = c \cdot (0.51 \cdot x_{S7} + 0.14 \cdot x_{S11} + 0.19 \cdot x_{S10} + 0.15 \cdot x_{S12}) + d$	0.73	0.40
3	$R = c \cdot \sqrt{x_{S8} \cdot x_{S10}} + d$	0.71	0.44				
4	$R = c \cdot (x_{18} + x_{S2} + x_{S6}) + d$	0.60	—	4w	$R = c \cdot (0.83 \cdot x_{18} + 0.0011 \cdot x_{S2} + 0.15 \cdot x_{S6}) + d$	0.70	0.55
5	$R = c \cdot (x_9 + x_{18} + x_{S2} + x_{S6})$	0.67	0.45	5w	$R = c \cdot (0.50 \cdot x_9 + 0.32 \cdot x_{18} + 0.0011 \cdot x_{S2} + 0.15 \cdot x_{S6}) + d$	0.70	0.45
6	$R = c \cdot \sqrt[3]{x_{18} \cdot x_{S4} \cdot x_{S6}}$	0.70	0.48				

<sup>a</sup> Six persons in the panel

<sup>b</sup>  $r$  = correlation coefficient

<sup>c</sup> M.D. = Mean difference between  $R_{cal}$  and  $R_{obs}$  when predicting unknowns (see text). The x-indices correspond to the peak numbers in Table 1.

**Table 4—Examples of best outcomes for the odor quality "burnt" when the models described have been applied to the 35 reference samples<sup>a</sup>**

Without weighting factors				With weighting factors			
No.	Model	$r^b$	M.D. <sup>c</sup> when predicting unknowns	No.	Model	$r^b$	M.D. <sup>c</sup> when predicting unknowns
7	$R = c \cdot (x_{S7} + x_{S10}) + d$	0.81	0.59	7w	$R = c \cdot (0.068 \cdot x_{S7} + 0.932 \cdot x_{S10}) + d$	0.89	0.58
8	$R = c \cdot (x_{32} - x_{17})/x_{12}$	-0.83	0.44				
9	$R = c \cdot \sqrt{x_{S4} \cdot x_{S10}} + d$	0.79	0.60	11w	$R = c \cdot (0.89 \cdot x_9 + 0.021 \cdot x_{S4} + 0.092 \cdot x_{S6}) + d$	0.78	0.85
10	$R = c \cdot \sqrt{x_{18} \cdot x_{S10}} + d$	0.82	0.60				

<sup>a</sup> Six persons in the panel

<sup>b</sup>  $r$  = correlation coefficient

<sup>c</sup> M.D. = Mean difference between  $R_{cal}$  and  $R_{obs}$  when predicting unknowns (see text). The x-indices correspond to the peak numbers in Table 1.



order to obtain the best fit to data when using any of the three basic models. This means that data were fitted by the method of nonlinear regression analysis in certain cases. A computer program performing this analysis was designed. The program calculates, as in the program for linear regression analysis, all possible peak combinations for every basic model and every odor quality. To perform this, iterative numerical methods had to be used. The models were ranked according to the correlation coefficients. Details about these models, the testing procedure and program are given by Åkesson et al. (1973).

RESULTS

WHEN THE MODELS were applied to the 35 reference samples, a great number of relations with high correlation coefficients were obtained, both with the unweighted and with the weighted models. Tables 3–8 show some of the outcomes for the odor qualities “retort flavor,” “burnt,” “sickly,” “sharp, pungent,” “meaty (cooked)” and “sulfurous.” Probable causative relations are placed below the dotted lines in the tables. It was evi-

dent that the weighted models have given an improved fitting to the data compared with the unweighted ones. For some odor qualities relations with  $r > 0.60$  were obtained only by using these models, e.g., for “sharp, pungent,” and “sulfurous.” The mean differences (M.D.) when predicting sensory properties of the “unknown” samples are also given in the tables. M.D. is the mean difference between the actual evaluation by the panel ( $R_{Obs}$ ) and the calculated flavor score ( $R_{Cal}$ ).  $R_{Cal}$  was computed by applying

Table 5—Examples of best outcomes for the odor quality “sickly” when the models described have been applied to the 35 reference samples<sup>a</sup>

Without weighting factors				With weighting factors			
No.	Model	r <sup>b</sup>	M.D. <sup>c</sup> when predicting unknowns	No.	Model	r <sup>b</sup>	M.D. <sup>c</sup> when predicting unknowns
12	$R = c \cdot (x_{17} / x_{63}) + d$	0.80	0.60	15w	$1nR = c \cdot 1n(0.28 \cdot x_{22} + 0.72 \cdot x_{S8}) + d$	0.81	0.54
13	$R = c \cdot (x_{17} - x_{63}) / x_{18} + d$	0.84	0.59	16w	$R = c \cdot (0.27 \cdot x_{22} + 0.44 \cdot x_{S8} + 0.29 \cdot x_{S10}) + d$	0.85	0.54
14	$R = c \cdot (x_{32} + x_{S5}) / x_{23} + d$	0.81	0.52				

<sup>a</sup> Six persons in the panel

<sup>b</sup> r = correlation coefficient

<sup>c</sup> M.D. = Mean difference between  $R_{Cal}$  and  $R_{Obs}$  when predicting unknowns (see text). The x-indices correspond to the peak numbers in Table 1.

Table 6—Examples of best outcomes for the odor quality “sharp, pungent” when the models described have been applied to the 35 reference samples<sup>a</sup>

With weighting factor			
No.	Model	r <sup>b</sup>	M.D. <sup>c</sup> when predicting unknowns
17w	$R = c \cdot (0.21 \cdot x_{13a} + 0.79 \cdot x_{S10}) + d$	0.93	0.40
18w	$R = c \cdot (0.021 \cdot x_{12} + 0.018 \cdot x_{22} + 0.063 \cdot x_{47} + 0.90 \cdot x_{S10}) + d$	0.91	0.44
19w	$1nR = c \cdot 1n(0.00035 \cdot x_{S2} + 1.0 \cdot x_{S10}) + d$	0.82	0.32
20w	$R = c \cdot (0.03 \cdot x_{S2} + 0.33 \cdot x_{S4} + 0.64 \cdot x_{S6}) + d$	0.79	0.41

<sup>a</sup> Six persons in the panel

<sup>b</sup> r = correlation coefficient

<sup>c</sup> M.D. = Mean difference between  $R_{Cal}$  and  $R_{Obs}$  when predicting unknowns (see text). The x-indices correspond to the peak numbers in Table 1.

Table 7—Examples of best outcomes for the odor quality “meaty (cooked)” when the models described have been applied to the 35 reference samples<sup>a</sup>

Without weighting factors				With weighting factors			
No.	Model	r <sup>b</sup>	M.D. <sup>c</sup> when predicting unknowns	No.	Model	r <sup>b</sup>	M.D. <sup>c</sup> when predicting unknowns
21	$1nR = c \cdot 1n x_{12} / x_{63} + d$	0.82	0.53	25w	$R = c \cdot (0.010x_4 + 0.97 \cdot x_{63} + 0.02x_{S2}) + d$	0.76	0.54
22	$R = c \cdot x_{S5} / x_{63} + d$	-0.74	0.30				
23	$R = c \cdot x_{32} / x_{23a} + d$	-0.72	0.24				
24	$R = c \cdot (x_{S5} + x_{S8}) / x_{63} + d$	-0.81	0.80				

<sup>a</sup> Six persons in the panel

<sup>b</sup> r = correlation coefficient

<sup>c</sup> M.D. = Mean difference between  $R_{Cal}$  and  $R_{Obs}$  when predicting unknowns (see text). The x-indices correspond to the peak numbers in Table 1.

Table 8—Examples of best outcomes for the odor quality "sulfurous" when the models described have been applied to the 35 reference samples<sup>a</sup>

With weighting factors			
No.	Model	r <sup>b</sup>	M.D. <sup>c</sup> when predicting unknowns
25w	$1nR = c \cdot 1n(0.010 \cdot x_{S6} + 0.99 \cdot x_{S10}) + d$	0.85	0.87
27w	$R = c \cdot (0.29 \cdot x_{S11} + 0.71 \cdot x_{S10}) + d$	0.86	0.75
28w	$R = c \cdot (0.46 \cdot x_{S7} + 0.54 \cdot x_{S10}) + d$	0.81	0.43
29w	$R = c \cdot (0.15 \cdot x_{S4} + 0.85 \cdot x_{S6}) + d$	0.88	0.95
30w	$R = c \cdot (0.015 \cdot x_{S2} + 0.99 \cdot x_{S4}) + d$	0.69	0.79
31w	$R = c \cdot (0.27 \cdot x_{S8} + 0.22 \cdot x_{S11} + 0.51 \cdot x_{S10}) + d$	0.86	0.76

<sup>a</sup> Six persons in the panel

<sup>b</sup> r = correlation coefficient

<sup>c</sup> M.D. = Mean difference between R<sub>cal</sub> and R<sub>obs</sub> when predicting unknowns (see text). The x-indices correspond to the peak numbers in Table 1.

chemical data from the "unknowns" to the regression models determined from the reference samples. For the majority of the relations M.D. was in the range 0.20–0.60, which is of the same order of magnitude as the standard deviations in R<sub>obs</sub>. Especially high correlations were obtained for the odor qualities "burnt," "sickly" and "sharp, pungent," some of them having correlation coefficients greater than 0.90.

It appears from the tables that there are several suggestions of causative relations, e.g., 2-methyl propanal (9), 2-methyl butanal (18), hydrogen sulfide (S2), methyl mercaptan (S4) and dimethyl sulfide (S6) were often included in the models for "retort flavor."

When predicting sensory properties for "unknown" samples there were no models working especially well for "sulfurous" (Table 8) and for the preference value. The M.D. values were in these cases in the range 0.8–1.5. The relative failure with "sulfurous" was probably due to the fact that this odor quality has been difficult to define unambiguously, the intensity having therefore a large standard deviation when assessed by the panel. Concerning the preference value, this cannot be expected to be monotonically related to chemical data in such a varied material as used in this investigation. The remaining sensory properties may be considered to be monotonically related to chemical data, which is a prerequisite for the models used.

A few other models showing high correlations for the reference samples did not work especially well when judging "unknown" samples. A reason for this may be that these models contain compounds which were technically hard to determine quantitatively.

## DISCUSSION

ONE OF THE REASONS for the successful results obtained in this investigation is

most likely the analytical methods used both on the chemical and the sensory side. The headspace technique used is gentle to the material and the sample represents in a more natural way the real situation of the food material than if an extraction or a distillation procedure had been used. Also, the analytical approach on the sensory side—the partition of the aroma into separate quality components—makes it easier to find monotonic relations.

The models introduced in the present investigations (Table 2) have been shown to work well, especially the weighted ones, and have resulted in improved fittings to the data. The geometric mean of the components occurred in several outcomes with high correlation coefficients, e.g., model no. 6 in Table 3, which seems to be a likely causative relation. The reasons why the geometric means including two, three or four compounds work well have been discussed by Persson et al. (1973b).

Introduction of the weighted models generally resulted in markedly higher correlation coefficients (Tables 3–8). A prerequisite of using these models is that they are tested in a large material since they contain additional free parameters. It must be pointed out that the weighted models shall be separated from the relations determined by a stepwise regression analysis procedure. The ML-estimation of the weighted relations gives the optimal outcomes of different combinations of compounds, e.g., the optimal combinations of three peaks are calculated and ranked according to goodness of fit, while the stepwise procedure gives only one outcome, which may not be an optimal estimation (cf. Åkesson et al., 1973).

It is interesting to note (cf. Tables 3–8) that the basic model (C):  $R = c \cdot S + d$ , occurred much more frequently than the logarithmic (A) or the exponential (B) basic models.

The models have been tested on a large material, thus resulting in predictive or causative outcomes rather than ad hoc relations. This was proved when relations determined from the reference samples were used to predict "unknowns." Only very few relations with high correlation did not work, showing that almost all the outcomes obtained were generally valid.

By applying the models in Table 3–7 the intensities of the various odor notes may be predicted instrumentally with the same accuracy as the panel's assessments. Examples of pure predictive relations are the outcomes placed above the dotted lines in Table 3, Table 4 and Table 6 and all the outcomes for the odor quality "meaty" (Table 7). For these there seem to be no suggestions of causative relations as, e.g., several compounds are present in too low concentrations compared with their odor thresholds. Examples of probable causative relations for the "retort flavor" are given in the lower half of Table 3. The compounds included in these relations are mainly the same as those suggested by Persson et al. (1973b) and it is confirmed that this off-flavor, at least in the first place, is caused by the methyl aldehydes, hydrogen sulfide, methyl mercaptan and dimethyl sulfide. For "sickly" it was difficult to choose relations that could be causative ones. Models nos. 12 and 13 in Table 5 might be considered as suggestions of causative relations. Model no. 20w for the odor quality "sharp, pungent" (Table 6) is e.g., a very evident suggestion of a causative relation. In Table 8, models nos. 29w and 30w seem to be of a causative nature. Examples of likely causative relations for the odor quality "burnt" (Table 4) are models nos. 9, 10 and 11w.

When discussing which components may be included in possible causative relations, only the odor properties, odor threshold values, concentration levels and sniffing data of the different compounds have been considered so far. However, other factors may also be of importance, e.g., the degree of change in concentrations of the compounds in the investigated material. Compounds having almost a constant concentration are of importance only as background stimuli. In addition, the aroma compounds have odor properties which are composed of different odor notes, e.g., hydrogen sulfide and methyl mercaptan have odor characters contributing with different degrees to the odor quality "burnt." To get an idea about the influence of these parameters on an odor quality it seems possible to use the weighted models.

In combination 2a in Table 2:  $S = w_i \cdot x_i + w_j \cdot x_j$ , the stimulus is composed of two terms:  $w_i \cdot x_i$  and  $w_j \cdot x_j$ . To evaluate the total contribution of each term to stimulation, it seems intuitively reasonable to estimate this by replacing  $x_i$  with

Table 9—The normalized "total weighting" values (see text) for the odor quality "burnt" calculated from the model  $R = c \cdot (w_i \cdot x_i + w_j \cdot x_j) + d$ . The x-indices correspond to the peak numbers in Table 1.

Normalized ( $w_{S6} \cdot (S_{S6})_{tot} = 100$ ) "total weighting" values			
$x_i$	$x_j$	Norm. $w_i \cdot (S_i)_{tot}$	Norm. $w_j \cdot (S_j)_{tot}$
9	S6	109	100
18	S6	59	100
17	S6	40	100
S2	S6	41	100
S4	S6	92	100

$(s_i)_{tot}$  and  $x_j$  with  $(s_j)_{tot}$ .  $(s_i)_{tot}$  and  $(s_j)_{tot}$  are the total standard deviations of the concentrations of compounds  $i$  and  $j$ , respectively, in this case a measure of the total variation of concentrations of  $i$  and  $j$  over the 35 investigated reference samples. Thus  $(s_i)_{tot}$  and  $(s_j)_{tot}$  are not a measure of the uncertainty of the gas chromatographic determinations. The total or integrated weight of the compound  $i$  is  $w_i \cdot (s_i)_{tot}$  and for compound  $j$ ,  $w_j \cdot (s_j)_{tot}$ . In Table 9 the total weights of nine individual compounds, which all were evaluated to contribute to the odor quality "burnt," have been calculated. Dimethyl sulfide (S6) has been included in one of the terms in all outcomes in Table 9. The "total weighting" values were then normalized by putting  $w_{S6} \cdot (S_{S6})_{tot} = 100$ . The values obtained in Table 9 may be used to calculate the weighting factors for the peaks when combined in three or four terms (3a and 4a in Table 2). The weighting factors determined in this way were of the same order of magnitude as the corresponding ones calculated directly by the computer program, which indicates that the values obtained in Table 9 are generally valid for the investigated material. At this stage, it thus seems possible that the compounds in Table 9 may be ranked after their importance for the odor quality "burnt" as follows: 2-methyl propanal (9) > dimethyl sulfide (S6) > methyl mercaptan (S4) > 2-methyl butanal (18) > 3-methyl butanal (17), hydrogen sulfide (S2). This list of precedence seems intuitively to be correct, e.g., dimethyl sulfide and methyl mercaptan have an odor character more resembling "burnt" than hydrogen sulfide.

For such a varied material as the one used in this investigation it cannot be expected that the preference value will be monotonically related to the chemical compounds, which was the case for the

more limited type of data in our investigations of stored frozen and refrigerated cooked beef (Persson and von Sydow, 1972). In the present investigation the preference value has been rated low both at very low and at very high concentrations of aroma compounds. The maximum value is situated somewhere between these two extremes (Persson et al., 1973a). With the weighted models a few outcomes were obtained, but they had low correlation coefficients and did not work very well when predicting unknowns. To relate chemical aroma data to sensory data meaningfully in such cases nonmonotone models ought to be used and work is going on developing such models.

As it has been shown above and by Persson and von Sydow (1972) it is possible to exclude ad hoc relations by repeating the experiments on independent materials. It would be desirable, however, to exclude such relations in a less laborious way. The presence of ad hoc relations is due to lack of exactness in the determination of the sensory intensities and/or the chemical concentrations. The latter may in its turn depend on analytical difficulties due to the method used or to the physical or chemical properties of the compounds. By improving the methodology in these regards it is possible to exclude compounds giving only ad hoc relations.

This investigation shows that the sensory properties, with the approach and models used, may be predicted by a gas chromatographic technique. This has also been shown to be the case at earlier investigations of stored cooked beef (Persson and von Sydow, 1972). The methodology is generally valid within the same type of food materials. Therefore, by applying the models in a proper way, the panel service in routine analyses may be supplemented or refined by using a gas

chromatographic technique. These methods may, of course, also be used in product and process development work to speed up the operation analysis procedure. In these cases causative rather than predictive relations should be used.

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## PRESSURE-INDUCED SOLUBILIZATION OF MEAT PROTEINS IN SALINE SOLUTION

### INTRODUCTION

SODIUM CHLORIDE (salt) is widely used in processed meat products. In addition to its effect on the flavor of these products, salt also affects such functional properties of meat proteins as water-holding capacity, emulsifying properties, and the texture or binding together of the cooked product (Kramlich, 1971; Trautman, 1966; Hamm, 1963; Swift, 1965; Turner and Olson, 1959; Wistreich, 1972). Its effects on the functional properties can be accounted for in terms of a swelling, as a result of an increase in hydration or in water-holding capacity (WHC), or a dissolution, of the myofibrillar proteins, although the mechanism by which this occurs is not fully understood.

Generally, hydration of proteins would be expected to result in an overall volume decrease in a system that contained proteins in an aqueous medium. This is because the effective volume occupied by a water molecule would be expected to decrease if the water molecule became more tightly bound. According to Le Chatelier's principle, an increase in pressure favors reactions that result in a decrease in the volume of a system and retards those that result in an increase in volume. Therefore it was considered that hydration of proteins and hence their WHC would be promoted by an increase in pressure. Moreover, studies reported by Marsland and Brown (1942) on myosin B, Josephs and Harrington (1967, 1968) on myosin, Ikkai and Ooi (1966) on actin, and Ikkai and Ooi (1969) on actomyosin indicate that the state of all of these important constituents of myofibrils can be influenced by the application of pressure. Therefore studies have been made of the effects of moderately high pressures on suspensions of sheep meat in saline solution to investigate whether the application of pressure could have technological significance. In the pressure studies referred to above, it was usually possible to make measurements on samples while they were under pressure. However, this was not possible in the investigations described here and the pressure effects reported are those that persisted after removal of the sample from the pressure cell.

### MATERIALS & METHODS

#### Meat source

The meat used throughout the experiments was obtained from 2-4 yr-old sheep. Following slaughter, carcasses were suspended from the Achilles tendon, and held at 2-4°C for 2-3 days before muscles were excised. Visible fat was trimmed from the muscles. Except where stated otherwise, the longissimus dorsi muscle was used.

#### Homogenates

Homogenates of meat in saline solution were prepared in the required weight ratios, usually 1:3 or 1:10, using an Ultra Turrax Mixer Emulsifier Type Tp 18/2 (Janke and Kunkel K.G. Staufen i. Br., W. Germany), and employing similar emulsification conditions at each weight ratio. The salt concentration in the saline solution was adjusted so that the aqueous phase of the homogenate was 0.5M NaCl assuming muscle to contain 75% salt-free water. When studies were done on the effects of variables such as duration of pressurization, meat concentration, or pH, treatment lots for each experiment were prepared from the same muscle. Before pressurization, homogenates were allowed to equilibrate overnight at 0°C.

#### pH measurement and adjustment

The pH of the homogenates was measured with a pH meter (Radiometer, Model 22) using a glass electrode. In order to determine the effect of pH on the response of homogenates to pressurization, the pH was altered by the addition of dilute HCl or NaOH during homogenization.

The pH reported is that measured the day following preparation of homogenates.

#### Pressurization

For pressurizing samples, either of two pressure vessels was used according to the amount of sample required. The chamber of one was 2.54 cm in diameter and 7.62 cm long, and of the other, 7.62 cm in diameter and 23.76 cm long. The working fluid in the small vessel was water and in the larger vessel, water and water-soluble oil in the ratio 64:1. The vessels were maintained at 0°C by surrounding them with an ice-water mixture.

For pressurization in the small vessel, about 10 ml of the well-mixed homogenate was placed in a glass test tube into which a liquid-tight stainless steel plunger with neoprene seals was fitted. For pressurization in the large vessel, the required quantity of homogenate was vacuum sealed in a polyethylene bag.

#### Expressed soluble protein (ESP)

To estimate changes in ESP, accurately weighed centrifuge tubes were filled with

homogenate, weighed again, and then centrifuged in a Beckman Spinco Preparative Centrifuge (Model L) at 35,000 rpm in a type 40.3 rotor (100,000 × G approximately) for 60 min. After spinning, each tube was inverted into a filter funnel lined with coarse tissue to remove fat particles and the decanted liquid was collected in a test-tube. After draining for 15 min, the tube with residue was weighed and the weights of the supernatant liquid and the residue calculated. Protein concentration in the liquid was determined using the biuret method of Gornall et al. (1949), and from this ESP was calculated as the % of the amount of protein originally present in the sample. Since a precise estimate of the total protein content of muscle was not required in order to characterize the reactions reported here, total protein was not measured but was assumed to constitute 20% by weight of the fresh meat (Young, 1970).

#### Water-soluble proteins

Water-soluble proteins were estimated by diluting the solutions used for ESP determinations until the salt concentration was approximately 0.05M. The precipitated salt-soluble proteins were removed by centrifuging and the water-soluble proteins remaining in the supernatant were estimated using the biuret method.

#### Water-holding capacity (WHC)

WHC was expressed as the weight of water associated with unit weight of protein estimated to be present in the residue obtained in the determination of ESP. For this estimation, meat was assumed to contain 20% by weight of protein and allowance was made for protein solubilized and consequently lost when liquid was decanted after centrifuging. Water content was estimated by measuring the weight loss produced in samples of known weight after heating in an oven at 105°C for 18-24 hr.

#### Column chromatography

Column chromatography of ESP samples was carried out using a Sepharose 4B packing (Pharmacia Fine Chemicals, Uppsala, Sweden) and a pH 6.5 NaCl-phosphate buffer as eluent, in a manner similar to that described by van Eerd (1972).

#### Cooking loss

Weighed samples of meat homogenates, each of 100g approximately, were cooked in sealed aluminum containers 7.6 cm in diameter and 2.7 cm deep for 30 min in a water bath controlled to 70 ± 0.5°C. Cooking loss was then estimated as the % loss of weight after the free liquid had been drained from the cooked sample by carefully tilting the opened container onto its side.

#### Young's modulus

Young's modulus (modulus of elasticity,

stress/strain) was measured on the drained cooked sample using an Instron Universal Testing Machine with a 2.26-cm diameter plunger.

## RESULTS

### Effect of pressure on WHC and ESP

In Figure 1(a) and (b) results are presented for the effect of pressurization on WHC and ESP respectively of muscles from seven animals. For meat samples taken from the one muscle, consistent effects of pressure on WHC were obtained in replicate experiments. However, from Figure 1 it can be seen that the effect on WHC varied between samples of the same muscle taken from different animals. For three samples, pressurization had little effect; for another three, WHC was found to have increased after pressurization up to a certain pressure, above which it decreased again. For the remaining sample, up to the highest pressure used, pressurization increased WHC. The ESP of all samples was found to have increased following pressurization at the highest pressures used, although at intermediate pressures there was an apparent decrease in ESP for two samples. This decrease in ESP may at least in part be due to an increase in the WHC of the sample which in turn resulted in a reduced quantity of supernatant liquid.

Other experiments were carried out using the meat from two animals, which will be referred to as A and B. Preliminary experiments revealed that the saline homogenate of meat from A showed a marked increase in WHC after application of  $69 \text{ MNm}^{-2}$  while that from B did not. Homogenates of meat from A and B were prepared in water without added salt. Both homogenates were centrifuged and found to have similar supernatant:sediment ratios. Salt was then added to each supernatant to give a 0.5M solution and the supernatant from A blended with the residue from B and vice versa. Investigation of the WHC of the homogenates after pressurization to  $69 \text{ MNm}^{-2}$  revealed that the residue from A retained a high WHC and that the supernatant from A did not confer a high WHC on the residue from B.

Homogenates prepared from each of five muscles (semimembranosus, semitendinosus, longissimus dorsi, psoas major and pectoralis) from one carcass were pressurized. The homogenates from all the muscles responded similarly, in the manner indicated by the open symbols on Figure 1(a) and (b).

### Nature of solubilized protein

Pressure was found to increase the yield of high molecular weight myofibrillar (salt-soluble) proteins relative to that of the low molecular weight (sarcolemmal) proteins. Results typical of those obtained are presented in Figure 2.

That pressurization resulted in an increased yield of salt-soluble protein can be seen also from the results contained in Figure 4(b) which are discussed in a later section. In this figure the open symbols (upper curves) indicate ESP yields which are composed of water- and salt-soluble

proteins. The solid circles (lower line) indicate the water-soluble protein content of the samples corresponding to the upper open circles. It can be seen that water-soluble protein yield was not markedly affected by pressurization and that pressurization could increase salt-

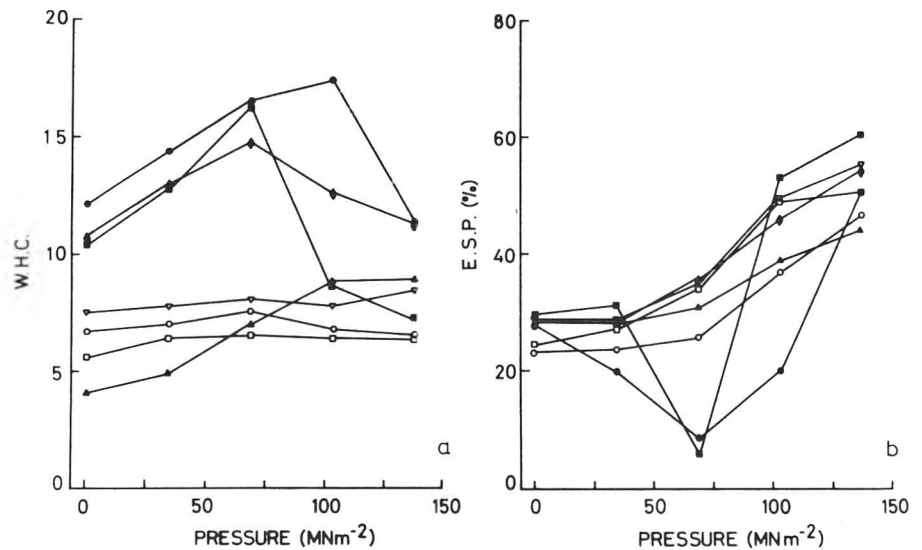


Fig. 1—Changes following pressurization in WHC (a) and ESP (b) of homogenates of meat (1 part) in sodium chloride solution (3 parts). Salt concentration in aqueous phase, 0.5M. Samples pressurized at  $0^\circ\text{C}$  for 5 min. Samples from different animals are indicated by different symbols.

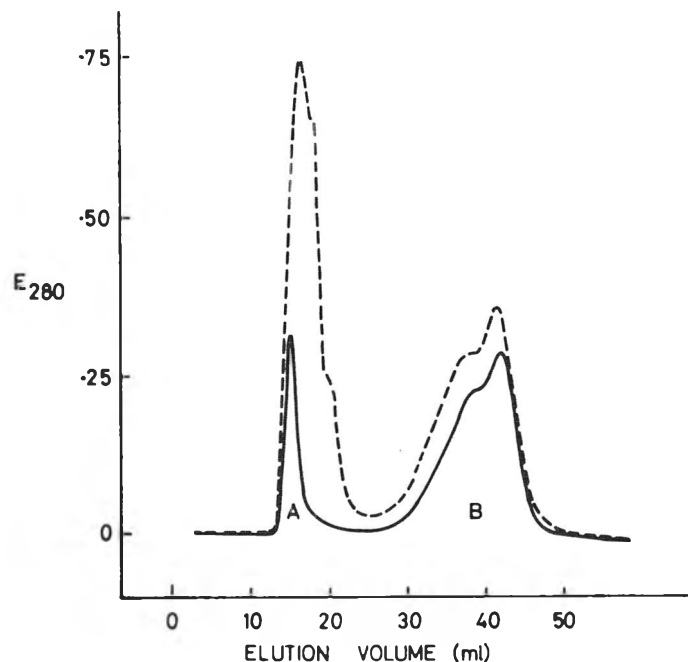


Fig. 2—Elution patterns of supernatant liquid obtained from nonpressurized (unbroken line) and pressurized (broken line) homogenates of meat in saline solution, after chromatography on Sepharose 4B. Pressurizing conditions:  $138 \text{ MNm}^{-2}$  for 5 min at  $0^\circ\text{C}$ . A: myofibrillar proteins; B: sarcolemmal proteins.

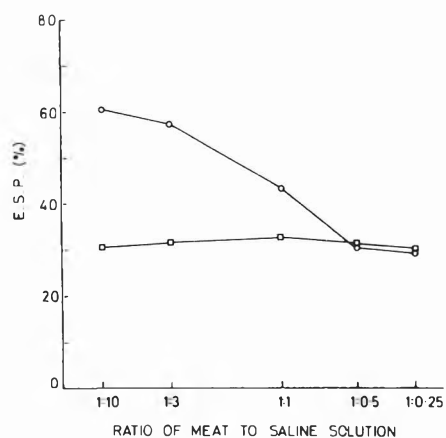


Fig. 3—Changes in the ESP of homogenates at various ratios of meat to sodium chloride solution. Salt concentration in aqueous phase, 0.5M. Circles: samples pressurized at 150  $MNm^{-2}$  for 5 min at 0°C; Squares: nonpressurized samples.

soluble protein yield up to about three-fold.

#### Cooking loss and Young's modulus

The effect of pressurization on the properties of meat proteins was further investigated by comparing the respective values for cooking loss and for Young's modulus of heat coagulated control samples and pressurized samples (Table 1). It can be seen that pressurization resulted in greatly reduced cooking losses but increased Young's modulus.

#### Meat concentration

The effectiveness of the pressurization treatment for the solubilization of proteins was dependent on the ratio of meat to saline solution in the sample pressurized (Fig. 3). Following pressurization, the ratio of meat to solution of all samples was adjusted to 1:10 by the addition of 0.5M saline solution and mixture homogenized then centrifuged. From Figure 3 it is seen that pressurization was ineffective for solubilizing protein at meat-to-saline solution ratios of 1:0.25 and 1:0.5, but its effectiveness rapidly improved as the ratio was decreased below unity.

#### Duration of pressurization

A meat-to-saline solution ratio of 1:10 instead of 1:3 was used in this and the investigations reported subsequently in order to achieve a better extraction of soluble protein. This also greatly reduced the influence of changes in WHC on the volume of the supernatant liquid obtained after centrifuging samples and hence on their ESP. In particular the decrease in ESP attributed to an increase in WHC as shown in Figure 1(b) was then not observed.

The effects of pressurizing were time-dependent, as can be seen from the results using pressures from 25 to 75  $MNm^{-2}$  [Fig. 4(a) and (b)]. For this sam-

ple WHC was markedly affected by pressurization. The results shown in Figure 4(a) suggest that the immediate effect of pressurization was to increase WHC. As

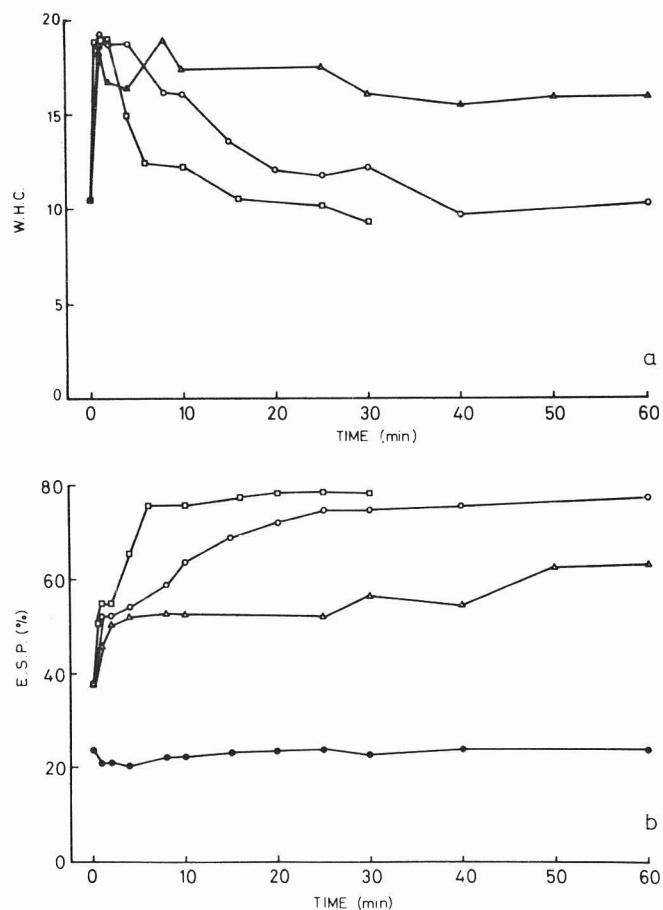


Fig. 4(a) and (b)—Open symbols: Changes with duration of pressurization in WHC and ESP respectively of homogenates of meat (1 part) in sodium chloride solution (10 parts), pH 5.6, concentration of salt in aqueous phase, 0.5M. Samples pressurized at 0°C. Squares, circles, and triangles: 75, 50 and 25  $MNm^{-2}$  respectively. Solid symbols: Water soluble protein yields from the nonpressurized and the 50  $MNm^{-2}$  treatments.

Table 1—Cooking loss and Young's modulus of heat coagulated meat-saline solution homogenates following various pressure treatments

Ratio Meat:saline solution	Pressure <sup>a</sup> $MNm^{-2}$	Cooking <sup>b</sup> loss, %	Young's modulus $\text{dynes cm}^2 \times 10^5$
1:3	0	44.3	1.04
	69	28.1	1.62
	138	6.7	1.48
1:1.5	0	33.6	2.08
	69	17.2	3.25
	138	3.0	4.45
1:0.5	0	1.9	12.0
	69	1.7	13.8
	138	1.1	14.3

<sup>a</sup> Samples pressurized for 5 min at 0°C

<sup>b</sup> Samples cooked for 30 min at 70°C

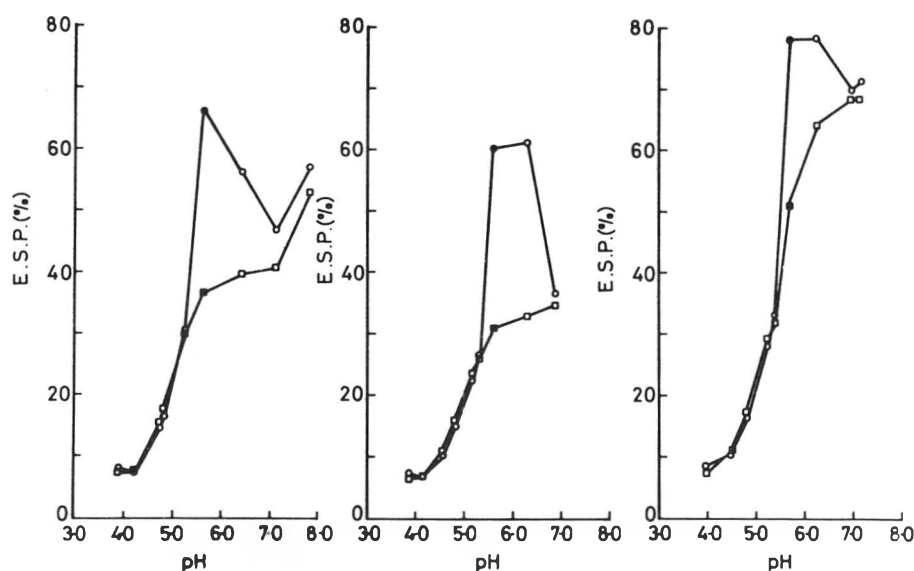


Fig. 5—Effect of pH on the ESP from homogenates of meat (1 part) in sodium chloride solution (10 parts), pressurized (circles) to  $138 \text{ MNm}^{-2}$  for 5 min at  $0^\circ \text{C}$  or nonpressurized (squares). The pH's of samples represented by the open symbols were adjusted using either HCl or NaOH solutions. Solid symbols show pH values of homogenates prior to adjustment of pH and pressurization. Each set of figures was obtained using meat from a different animal.

the duration of pressurization was increased, WHC decreased and ESP increased. Even the lowest pressure used,  $25 \text{ MNm}^{-2}$ , increased the WHC, although at this pressure the subsequent solubilization of proteins was relatively slow. This figure also shows the water-soluble protein yield obtained from the nonpressurized and the  $50 \text{ MNm}^{-2}$  treatments. As mentioned earlier, the pressure treatment had little effect on the yield of these proteins.

#### Effect of pH

The effect of pressurization is also dependent on the pH of the homogenates, as indicated in Figure 5. The pH values of homogenates measured before and after pressurizing were found to be similar. However, it is noted by Johnson et al. (1954) that pressure affects the degree of ionization of buffer systems. Therefore the pH of a homogenate while under high pressure probably differs from the pH when under atmospheric pressure. Data in Figure 5 reveal that pressure did not effect yields of ESP until pH exceeded 5.3, and the main effect occurred in the range pH 5.3–7.

#### Pressure effects during centrifuging

During the analysis for ESP, samples were centrifuged at high speed (35,000 rpm) for 60 min. It was calculated that at the bottom of the centrifuge tubes, pressures of the order of  $30 \text{ MNm}^{-2}$  could be developed. In view of the results presented above, it seemed possible that centri-

fuging itself may increase the yield of salt-soluble proteins. To investigate this possibility, samples of homogenate were centrifuged for the same time but at different speeds ranging from 35,000–5,000 rpm. Because the pressure developed in the centrifuge tubes is proportional to angular velocity squared,

the pressures developed at the lower speeds were relatively very small and therefore unlikely to affect protein solubility. After centrifuging, changes in the protein concentration in the supernatant fluid were investigated. The results presented in Figure 6 show that increasing the speed of centrifugation above 10,000 rpm resulted in a slight decrease in the protein concentration of the supernatant. Thus there was no indication of increased solubility of protein at the higher speeds or at least, any increase in solubility was more than compensated for by a decrease in the protein content of the fluid released at the higher speeds.

#### DISCUSSION

JOHNSON et al. (1954) state, "In general there are at least two, and possibly four, mechanisms involving volume change through which high pressures would be expected to influence the stability of proteins. They are: by affecting (i) the amount of ordinary ionization or of zwitterion formation, (ii) the process of unfolding following the weakening of hydrogen bonds, (iii) the structure of the solvent, and (iv) the degree of ionization of a buffer system."

Of these mechanisms, those involving ionization, i.e., (i) and (iv), appear likely to be of greatest importance for the present study. Johnson et al. (1954) mention that mechanism (ii) is likely to involve an increase in volume. Therefore reactions involving this mechanism would be opposed by pressure unless outweighed by associated reactions involv-

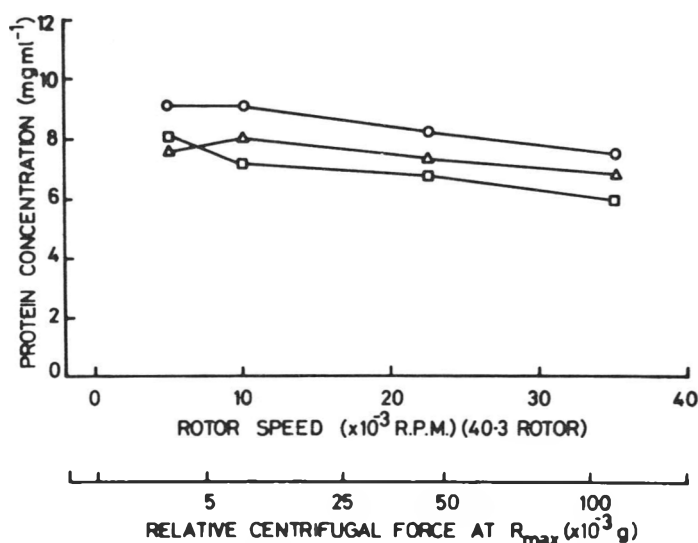


Fig. 6—Protein concentration of the supernatant liquid from homogenates (meat/saline solution = 1:10) centrifuged at the rotational speeds indicated for 1 hr at  $0^\circ \text{C}$ . pH values of samples: A, 5.57; B, 5.60; C, 5.65.

ing a volume decrease. When water is the solvent, mechanism (iii) is probably only of significance at very high pressures (Johnson et al., 1954).

The effect of salt on the WHC of meat has been discussed by Hamm (1960). Above the isoelectric point salt is thought to cause an increase in the WHC of meat as a result of a loosening of protein structure leading to increased hydration. An ionic mechanism is proposed and because of electrostriction effects between water molecules and exposed ions, the reaction would be expected to occur with a decrease in volume. Therefore this type of reaction should be favored by application of pressure and this is consistent with the pressure-induced increase in WHC observed in some samples as shown in Figure 1(a).

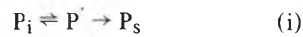
Examination of the results presented in Figure 1(a) suggests that meat can be broadly divided into two types according to whether pressure has a marked or only a slight effect on WHC. From the experiments in which the residues and the supernatants from each type of meat were exchanged, it appears that the factor determining the type of pressure-WHC effect is not present in the water soluble fraction of meat. It was noted too, that generally those samples whose WHC was increased by pressurization also had a relatively high WHC when not pressurized. The reasons for the observed differences between samples in their WHC following pressurization have not been investigated further.

At sufficiently high pressures [Fig. 1(b)], or with prolonged pressurization [Fig. 4(b)], salt-soluble protein in all six muscles was solubilized.

The results presented in Figure 4(a) and (b) suggest the occurrence of a consecutive reaction: protein  $\rightarrow$  protein of increased WHC  $\rightarrow$  solubilized protein. At the lowest pressure investigated, 25 MNm<sup>-2</sup>, the change was relatively slow as evidenced by the gradual increase in ESP after an initial relatively small, but rapid, rise. At 75 MNm<sup>-2</sup> the change was much faster, ESP values changing little if the duration of pressurization was longer than 5 min.

The rate of the pressure-induced reaction may be dependent on many factors, e.g., meat concentration, pH, temperature during pressurization, method of homogenizing sample, etc. Investigation of the effect of all these factors on reaction rate was outside the scope of the present investigation. However it should be noted that in most of the investigations reported here the duration of pressurization was 5 min. Therefore, particularly at pressures in the lower part of the pressure range investigated, the pressure-induced reactions reported may not have reached equilibrium when pressurization was

terminated. The effect of pressure on chemical reaction rates has been discussed by Johnson et al. (1954). The effects described above, where the pressure-induced reaction is apparently irreversible, appear to be consistent with a pressure-induced reaction occurring via an activated intermediate:



where  $P_i$  = protein insoluble in saline solution before pressurization;  $P'$  = activated intermediate reversibly formed from  $P_i$ , but increasing pressure shifts the equilibrium towards  $P'$ ; and  $P_s$  = solubilized proteins produced from  $P'$  by an irreversible reaction.

If the reaction is of the type indicated by equation (i), then from the change in reaction rate with pressure it should be possible to calculate the volume change,  $\Delta V^\ddagger$ , involved in the formation of the activated intermediate using the equation:

$$k_p = k_0 e^{-p \Delta V^\ddagger / RT} \quad (ii)$$

(Johnson et al., 1954) where  $k_0$  is the rate at zero pressure,  $k_p$  is the rate under pressure  $p$ ,  $\Delta V^\ddagger$  is the volume of the activated complex minus the volume of the reactants,  $p$  is the pressure in atmospheres,  $R$  is the gas constant and  $T$  is the absolute temperature. However the scatter of experimental values about rate curves of the type shown in Figure 4(b) was too great to allow useful analysis of the shape of the curve in order to apply equation (ii).

From the data presented in Table 1 on cooking loss and Young's modulus, it appears that the pressure solubilized proteins retained useful functional properties. When heat coagulated, they were effective in holding water and binding together. However, inspection of the results presented in Figure 3 reveals that the pressurization treatment was ineffective for solubilizing protein when the ratio of meat to solution was high and this might restrict the technological usefulness of the effect.

From the results presented in Figure 5, pressurization appears to be completely ineffective for promoting the solubilization of protein from pH 5.3 down to the lowest pH investigated, 3.9. Studies of the effect of pH on the response of homogenates to pressurization are complicated by the fact that the application of pressure itself changes the pH (Johnson et al., 1954). However any pH change that does occur appears to be reversible as following the release of pressure, the pH of samples was the same as their initial value. Hamm (1960) reported that at least over the pH range 4.5–7.0, the influence of pH on muscle hydration was reversible. Therefore provided that the

pH remained within these limits, changes in hydration, observed here as changes in WHC, are not likely to be the result of pH changes produced by pressure.

Although the isoelectric point ( $I_p$ ) of fresh meat is around 5.0, it is lowered in the presence of salt (Hamm, 1960) and in these experiments is probably close to pH 4.0 since protein solubility appears to be near a minimum in this region (Fig. 5). Hamm classified pH-hydration curves into several types. Comparison of the ESP-pH curve reported here with these types reveals greatest resemblance with type 1 change reported by Hamm, i.e., no change in  $I_p$  and ESP increases in the basic range of  $I_p$ . However the pressure-induced increase in ESP was not observed over the whole pH range on the basic side of the  $I_p$ , not being apparent until pH > 5.3 and being doubtful at pH 7 and above. In considering reasons why the pressure solubilizing effect should be restricted to this range, it was noted that it corresponds to the titration of imidazole groups (Mahon, 1961) suggesting the involvement of these groups in the pressure effect.

Josephs and Harrington (1967) reported that the pressures developed in samples in the ultracentrifuge affect the association of myosin molecules. Likewise, it seemed possible that the pressures developed in samples when centrifuged under the conditions used here could induce changes of the type under study. However, no evidence for the existence of an effect on protein solubility was found, as reported in the results from samples centrifuged at different rotational velocities (Fig. 6).

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## MUSCLE CATHEPSINS IN THREE SPECIES OF PACIFIC SOLE

### INTRODUCTION

RECENTLY, considerable attention has been given to the autolytic deterioration of fish tissue. Although a major source of fish spoilage is microbial (Liston, 1965), it is well known that proteolytic enzymes occupy an obscure role in the degradative process (Siebert, 1962; Siebert and Schmitt, 1965; Dolla and Blackwood, 1962). Siebert (1962) found autolytic activity in fish muscle which was capable of hydrolyzing up to 10 mg of protein per hr per g of fresh muscle tissue. The autolysis of fish muscle postmortem by proteolytic enzymes may be important in utilizing and preserving marine food products (Makinodan and Ikeda, 1969b).

Cathepsins have their highest activity when exposed to low acidic conditions (ca. pH 4.0) (Fruton, 1960) but their fundamental role remains unknown as the pH in most fish muscle seldom declines lower than 6.2 (Buttkus and Tomlinson, 1966).

Siebert (1958) and Siebert and Schmitt (1965) reported a variation in the catheptic activity of animal tissue and that fish contained a higher proteolytic activity (ca. 10 times) than mammals. In later studies Wojtowicz and Odense (1972) reported comparable catheptic activity in chicken muscle to that in cod muscle.

Since there has been increased speculation about the spoilage effect that cathepsins may exert upon the postmortem quality of fish muscle, an investigation was undertaken with three species of Pacific sole to further explore the validity of this assumption. The species included rex (*Glyptocephalus zachirus*), English (*Parophrys vetulus*) and petrale (*Eopsetta jordani*) sole.

Partial characterization of the muscle cathepsins in the three species of sole provided information on optimum assay conditions for determining comparative activity. Sensory evaluations were conducted to determine if judges could distinguish a difference between fish muscle of the same species containing different levels of catheptic activity.

### EXPERIMENTAL

ENGLISH (*Parophrys vetulus*), rex (*Glyptocephalus zachirus*) and petrale (*Eopsetta jordani*) sole were obtained in the round from

commercial fishermen or fish processors located at Winchester Bay or Astoria, Oregon. The fish were filleted in such a manner to allow the removal of the lateral line which contained small amounts of dark muscle and to separate the top fillet (dark side of whole sole) from the bottom fillet.

#### Preparation of enzyme extracts

The assay technique used was a modification of the procedure outlined by Wojtowicz and Odense (1972). 50g of white muscle tissue from the top fillet of the sole was homogenized (1:5) with 250 ml of 1% NaCl at high speed for 2 min in an Osterizer blender. The homogenate was frozen and held at  $-26^{\circ}\text{C}$ . Prior to assay, the frozen homogenate was slowly thawed by placing the container in a cold water bath. The thawed homogenate was centrifuged at  $10,000 \times G$  for 20 min at  $4^{\circ}\text{C}$ . The extract (supernatant) was used as the enzyme source for the succeeding experiments. The particulate was discarded.

#### Preparation of hemoglobin substrate

A 2.5% buffered hemoglobin solution was prepared by the addition of approximately 450 ml of 0.2M glycine-HCl buffer (pH 3.0) to 12.5g of native hemoglobin (Matheson, Coleman and Bell) and 12.5 mg of Thimerosal (sodium ethylmercurithiosalicylate) preservative. The mixture was dissolved by stirring at room temperature and the pH of the solution adjusted to 3.0 with 6N HCl. The solution was diluted to 500 ml with buffer, centrifuged at  $10,000 \times G$  for 10 min to remove undissolved hemoglobin and the resulting solution stored at  $4^{\circ}\text{C}$ .

#### Assay procedure

2 ml of saline enzyme extract was added to a test tube (18  $\times$  150 mm) containing 3 ml of 2.5% buffered hemoglobin solution (pH 3.0) that was preincubated in a water bath at  $35^{\circ}\text{C}$  for 5 min. The reaction was terminated after 1 hr incubation at  $35^{\circ}\text{C}$  by the addition of 5 ml of 10% trichloroacetic acid (TCA). After standing for at least 30 min, the reaction mixture was filtered through Whatman No. 3 filter paper. Released "tyrosine-like compounds" in the filtrate were determined spectrophotometrically at 660 nm against a distilled water blank according to the method of Lowry et al. (1951). Assays were carried out in duplicate or triplicate. To allow for substrate hydrolysis, controls containing only hemoglobin were incubated in a similar manner as the samples with addition of TCA and enzyme extract, respectively, after the appropriate incubation period. Activity was expressed as the  $\Delta A_{660 \text{ nm}} / \text{cm}^2 / \text{g muscle/hr}$  according to the conditions specified.

#### Freezer storage of saline muscle homogenates

A 25 day frozen storage trial was carried out

on duplicate homogenates of English sole to determine the influence of frozen storage on catheptic activity. Two (1:5) saline homogenates of muscle from two English sole were each divided into six equal aliquots. Five aliquots from each sole were immediately stored at  $-26^{\circ}\text{C}$  and the hemoglobin-splitting activity of the two remaining aliquots (one from each sole) which served as zero time samples was determined. At 5-day intervals an aliquot of the homogenate from each fish was removed, thawed, centrifuged and the extract assayed for hemoglobin-splitting activity.

#### Partial characterization

The catheptic activity of muscle extracts from rex, English and petrale sole was determined to obtain optimum assay conditions. The hemoglobin-splitting and autolytic activity was determined as a function of incubation time. Autolysis was defined as the hydrolysis of the soluble protein that was contained in the extract which served as the enzyme source. 3 ml of 0.2M glycine-HCl buffer (pH 3.0) containing the appropriate amount (1.0 mg/40 ml solution) of Thimerosal preservative served as a substitute for hemoglobin in assays for autolytic activity.

Catheptic activity as a function of temperature was determined at temperature increments of  $10^{\circ}$  from  $15-65^{\circ}\text{C}$ .

The desired substrate pH for determining the pH of optimum activity was achieved by adjusting the pH of aliquots of a 2.5% buffered hemoglobin solution (0.2M glycine-HCl; pH 2.8 or 3.6) with 6N HCl or NaOH.

In the assay for determining catheptic activity as a function of enzyme concentration, the total volume of reactants was increased to 10 ml. The volume of extract varied from 0.5 to 5 ml while the volume of hemoglobin substrate remained constant at 5 ml. With the exception of the sample containing 5 ml of enzyme extract, 1% NaCl served to bring the final reaction volume to 10 ml. The volume of 10% TCA used to terminate the reaction was increased proportionally.

The catheptic activity as a function of hemoglobin concentration was determined only for muscle extracts of rex and English sole. An initial 5% buffered hemoglobin solution (pH 3.0) was prepared according to the method previously described and serial dilutions of 0.1, 0.5, 1.0, 1.5, 2.0, 2.5 and 4.0 (%) were made by adding appropriate amounts of buffer. The buffer served as a hemoglobin substitute for activity measurements at zero percent substrate concentration.

#### Comparative catheptic activity

Samples of rex, English and petrale sole (25 individuals of each species) were obtained from a commercial fishing boat off the north coast of Oregon. The Pacific sole were all taken from the same tow and immediately separated by species at sea, stored in crushed ice and

designated as zero days storage. In part on the following day, the samples were treated with additional ice and stored at 4°C for an additional 3 days. After a total of 4 days storage, the fish were removed, filleted, homogenates prepared and hemoglobin-splitting activity determined according to the previously described method. Remaining muscle tissue from both top and bottom fillets of individual sole were vacuum sealed together in moisture-vapor proof film and frozen at -26°C. The excess muscle tissue was marked in such a manner as to be identified with its respective homogenate. The packaged frozen muscle was used for sensory evaluations.

Sensory evaluation

From the data on the comparative catheptic activity in rex, English and petrale sole muscle extracts, arbitrarily selected values were chosen for each individual species to represent a high and low activity range. After establishing a difference range in catheptic activity, several packages (depending upon their weight) of the premarked, frozen muscle tissue of each species were evaluated by the triangle difference test.

The fish muscle samples were prepared under similar cooking conditions by placing them in boiling water in their respective moisture-vapor proof film package. After limited cooking to preserve firm muscle texture, the samples which represented the appropriate catheptic activity range groups of the three species were flaked into composite samples and served in coded cups to 30 untrained judges seated in individual booths.

RESULTS & DISCUSSION

Frozen storage of saline muscle homogenates

The catheptic activity of two muscle extracts (x and y) from English sole during a 25 day frozen storage period at

Table 1—Effect of frozen storage (-26°C) on the catheptic activity in saline extracts of English sole muscle

Days in frozen storage	Activity ( $\Delta A_{660 \text{ nm}}^{1 \text{ cm}} / \text{g muscle/hr}$ )	
	x	y
0	8.59	2.30
5	8.59	2.38
10	9.04	2.65
15	8.84	2.57
20	8.86	2.54
25	8.15	2.41
Mean (x)	8.68	2.48
Std dev ( $\sigma$ )	0.31	0.13
Std error	0.13	0.05

-26°C remained relatively constant as shown by their respective standard deviation of 0.31 and 0.13 (Table 1). This result corroborated the findings that Groninger (1964) obtained with a purified albacore extract, but indicated a difference from the properties of bovine muscle cathepsins. Parrish and Bailey (1967) found increased catheptic activity in granules of bovine muscle after freezing.

Partial characterization

The catheptic activity of muscle extracts from rex and English sole toward hemoglobin and endogenous muscle proteins as a function of incubation time closely paralleled each other (Fig. 1 and 2). The soluble proteins found in the extract were referred to as endogenous

muscle proteins. The hemoglobin-splitting activity appeared linear during the 3-hr incubation period with regression analyses providing correlation coefficients of 0.998 for rex and 0.992 for English sole. The autolytic rate for both species (rex and English) of sole was linear during the first 2.5 hr of incubation with a slight decline in activity observed for the remaining 0.5 hr of the 3-hr incubation period. This digression from linearity for autolytic activity after 2.5 hr of incubation suggested that substrate concentration may have become a limiting factor.

The hemoglobin-splitting activity of the muscle extract from petrale sole was linear in relation to time during the entire 3-hr incubation period. Regression analysis provided a correlation coefficient of 0.998 (Fig. 3). After an initial lag period

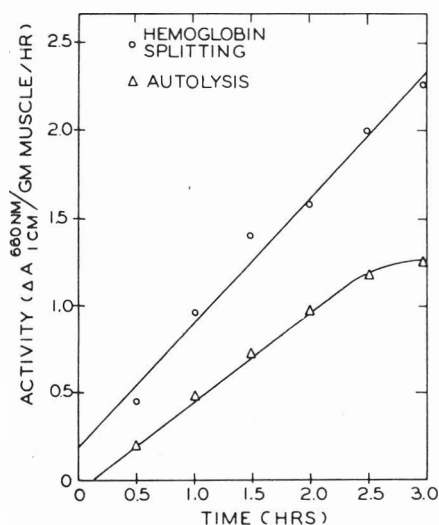


Fig. 1—Catheptic activity of a muscle extract from rex sole toward hemoglobin and endogenous muscle proteins as a function of incubation time (35°C; pH 3.2).

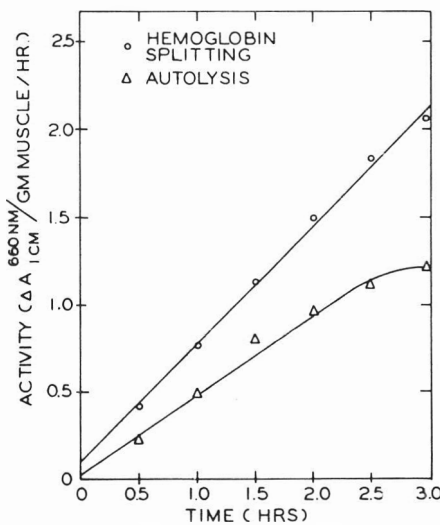


Fig. 2—Catheptic activity of a muscle extract from English sole toward hemoglobin and endogenous muscle proteins as a function of incubation time (35°C; pH 3.2).

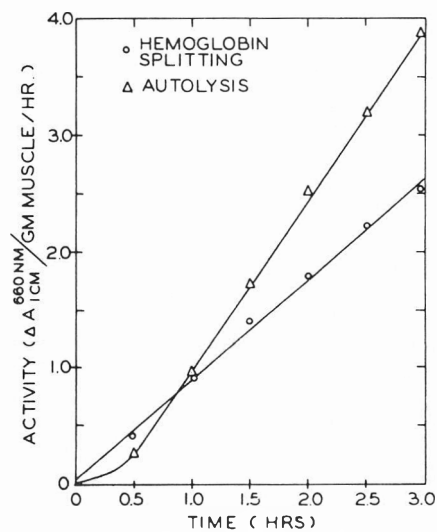


Fig. 3—Catheptic activity of a muscle extract from petrale sole toward hemoglobin and endogenous muscle proteins as a function of incubation time (35°C; pH 3.3).

the autolytic rate was virtually linear during the 3-hr incubation period and achieved a rate exceeding that observed for hemoglobin-splitting.

Using a bovine albumin standard colorimetric curve (Lowry et al., 1951), the petrale sole muscle extracts were shown to contain a larger amount of soluble protein than the muscle extracts of either rex or English sole. The higher autolytic activity in relation to hemoglobin-splitting activity observed in petrale sole as compared to either rex or English sole

could have been related to either substrate concentration and/or composition. Extracts of petrale sole may have contained higher levels of soluble proteins which were more susceptible to hydrolysis.

All three species of sole showed a catheptic activity temperature optimum at 45°C (Fig. 4). Groninger (1964) reported a temperature optimum of 42°C for a purified aqueous extract of albacore muscle and Makinodan and Ikeda (1969a) reported similar findings in several species

of fish. However, a temperature optimum of 35°C was reported by Saitō and Sameshima (1958) for extracts of carp, mackerel and shark.

A pH of 3.0–3.5 was found to be optimum for the catheptic activity in muscle extracts of rex and petrale sole, but a slightly higher pH optimum range of 3.2–3.8 was observed in English sole (Fig. 5). This pH optimum was in agreement with several other investigators who characterized acidic proteases in various fish specimens (Saitō and Sameshima, 1958; Makinodan and Ikeda, 1969a; Dollar and Blackwood, 1962; Wojtowicz and Odense, 1972; Ting et al., 1968). Groninger (1964) found a slightly more acidic pH optimum (2.4–2.5) for a protease from the muscle of albacore tuna. Purified cathepsin from cod muscle was reported by Siebert (1962) to have a pH optimum near 4.3. Reddi et al. (1972), in an investigation of muscle cathepsins in winter flounder, a member of the flatfish family, found a pH optimum of 4.0.

A linear relationship was shown to exist between activity and enzyme concentration in the muscle extracts of all three species of sole (Fig. 6) as indicated by correlation coefficients of 0.997, 0.991 and 0.995 for rex, English and petrale sole, respectively. The ratio of reagents in the assay containing 3 ml of enzyme muscle extract in this experiment was virtually equivalent to the ratio used in the original assay procedure. The results shown on Figure 6 indicated that enzyme concentration was a limiting factor as desired in the assay procedure.

The assay system appeared saturated with substrate near a hemoglobin concentration of 1.0% (Fig. 7) and a progressive inhibitory effect was observed at concentrations greater than 2.0%. An insufficiently hydrated medium for protein-water interaction may explain this reduction in hemoglobin-splitting ability. It appeared that only relatively small quantities of exogenous protein were necessary to saturate the assay system.

From the enzyme kinetic data, the original assay procedure was modified to achieve optimal assay conditions for the subsequent comparative activity investigation. The incubation temperature was increased from 35°C to 45°C and the concentration of hemoglobin was decreased from 2.5% to 1.5%.

#### Comparative catheptic activity

A considerable variation within individuals was observed for rex sole as was statistically revealed by a catheptic activity range of 17.21 and a standard deviation of 4.70 from a mean of 4.15 (Table 2). English and petrale sole showed a relatively more uniform pattern with catheptic activity ranges of 4.70 and 1.55, respectively. English sole with a standard deviation of 1.24 from a mean

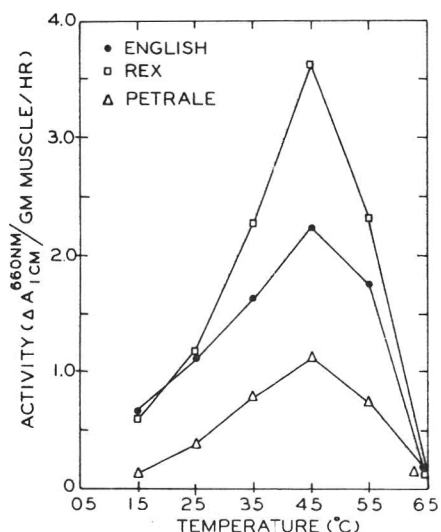


Fig. 4—Catheptic activity of a sole muscle extract toward hemoglobin as a function of incubation temperature (pH 3.2–3.3).

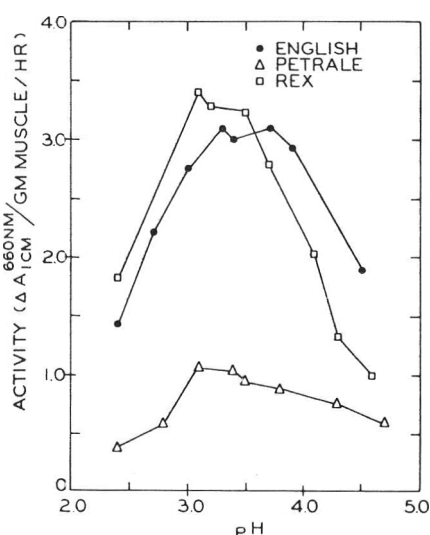


Fig. 5—Catheptic activity of a sole muscle extract toward hemoglobin as a function of pH (35°C).

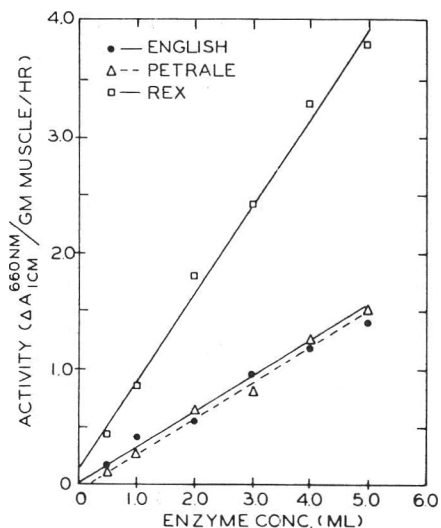


Fig. 6—Catheptic activity of a sole muscle extract toward hemoglobin as a function of enzyme concentration (35°C).

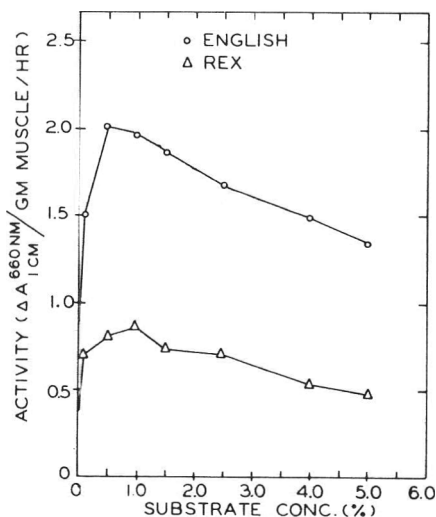


Fig. 7—Catheptic activity of a sole muscle extract toward hemoglobin as a function of substrate concentration (35°C; pH 3.3).

**Table 2—Catheptic activity of muscle extracts from three species of Pacific sole toward 1.5% hemoglobin (45°C; pH 3.2–3.3)**

	Activity ( $\Delta A_{1\text{ cm}}^{660\text{ nm}}$ /g muscle/hr)		
	rex	English	petrale
Mean ( $\bar{x}$ ) <sup>a</sup>	4.15a	3.45ab	1.74b
Std dev ( $\sigma$ )	4.70	1.24	0.49
Range	17.21	4.70	1.55
Median	2.14	3.22	1.59

<sup>a</sup> n = 23; Mean activities with like letters did not vary significantly (P < 0.05) from each other.

**Table 3—Triangle difference tests on muscle from same specie which contained different levels of catheptic activity**

Statistics	Specie		
	rex	English	petrale
No. of individual samples at each activity level <sup>a</sup>	8	5	3
Activity difference:			
High: range	13.48	1.82	0.22
mean	9.25	5.19	2.50
std dev	4.73	0.71	0.13
Low: range	0.52	0.58	0.04
mean	0.84	2.04	1.15
std dev	0.20	0.27	0.02
Total no. of judgements	30	30	30
No. of correct judgements <sup>b</sup>	13	10	15
Difference (P < 0.05)	NS	NS	NS

<sup>a</sup> Weight of fillet with correspondent catheptic activity dictated the needed sample number.

<sup>b</sup> Number of correct judgements needed to be significant: 16 (P < 0.05) or 18 (P < 0.01).

of 3.45 was shown to vary more than petrale sole with a standard deviation of 0.49 from a mean of 1.74.

Analysis of variance for the catheptic activity in the three species of sole showed a significant difference between their mean values (F-value, 4.48). Duncan's multiple range test revealed that the mean activity in muscle of rex and petrale sole varied significantly, while the mean activity in muscle of English sole did not vary from that of either rex or petrale sole.

Conclusions drawn from the above results were restricted to the specified conditions, i.e., assay of 23 individuals of each species which were taken from the same tow. If influencing factors such as season, location, feed, age, sex and

spawning were considered, the relationships observed could change. However, the results indicated that variation in catheptic activity existed between and within species.

**Sensory evaluation**

Using a triangle difference test, a flavor panel of 30 judges found no significant difference in the muscle of petrale, rex or English sole that contained high and low levels of catheptic activity with 15, 13 and 10 correct judgements, respectively (Table 3). Results of sensory evaluation did not support a significant degradative role for catheptic enzymes in the muscle of round fish that were stored for a period of 4 days post-catch on ice. This observation further necessitates addition-

al investigation of cathepsins for their role and/or function in fish muscle post-mortem.

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G. M. SAPERS, O. PANASIUK, S. B. JONES, E. B. KALAN and F. B. TALLEY  
 USDA Eastern Regional Research Center, Northeastern Region, ARS, Philadelphia, PA 19118  
 and R. L. SHAW  
 Red River Valley Potato Research Center,<sup>1</sup> Box 113, East Grand Forks, MN 56721

## IRON FORTIFICATION OF DEHYDRATED MASHED POTATOES

### INTRODUCTION

DIETARY IRON deficiency has been recognized as a significant nutritional problem in the United States (White House Conference on Food, Nutrition and Health, 1970). One approach to the solution of the problem is the addition of iron to foods consumed by large segments of the population. The feasibility of fortifying whole milk with various iron compounds was reported recently by Edmondson et al. (1971).

The high per capita consumption of potatoes (118 lb in 1970), the increasing acceptance of dehydrated potato products (USDA, 1971) and their inclusion in the U.S. Department of Agriculture

Needy Families Program make this food a potentially suitable vehicle for iron fortification. One commercial producer of potato flakes is enriching his product with "iron phosphate," but the level of addition (6.8% of the recommended dietary allowance for a 22-yr-old man in one 4 oz. serving) is probably too low to have much nutritional impact. The desirability of adding higher levels of iron must be reconciled with the possible occurrence of "after-cooking darkening," a discoloration resulting from the formation of iron-chlorogenic acid complexes (Burton, 1966) and with the catalysis of oxidative rancidity during storage (Ingold, 1962).

Exploratory studies were undertaken at Eastern Regional Research Center (ERRC) to determine the technical feasibility of fortifying dehydrated mashed potatoes with nutritionally significant levels of iron. The results of these studies are reported herein.

### EXPERIMENTAL

#### Iron compounds and levels of fortification

Dehydrated mashed potatoes were fortified with a number of commercially available iron compounds (reagent or U.S.P. grade) as well as with several experimental iron complexes developed at ERRC (Table 1). The latter have been described by Jones et al. (1971, 1972).

Compounds were tested at three levels corresponding to 2, 5 and 10 mg iron per 100g mashed potatoes. The highest level represents approximately 55% of the recommended daily dietary allowance for pregnant and lactating women (NRC, 1968).

#### Effect of iron compounds on the color of mashed potatoes

Since the induction of after-cooking darkening was believed to be the most serious obstacle to iron fortification of potato products, iron compounds were screened initially on the basis of color formation in mashed potatoes.

Iron compounds were added in aqueous solution or as finely ground powders to 100g portions of fresh mashed potatoes, prepared

<sup>1</sup> A center cooperatively operated by the USDA North Central Region, ARS; the Minnesota Agricultural Experiment Station; the North Dakota Agricultural Experiment Station; and the Red River Valley Potato Growers' Association.

Table 1—Discoloration of iron fortified mashed Idaho Russet potatoes

Iron compound added	Iron content (%)	Relative biological value (%)	Mashed potato color			
			mg Fe/100g			
			0	2	5	10
Ferrous sulfate	20.0	100 <sup>a</sup>	Normal	Dark	Dark	Dark
Ferrous ammonium sulfate	14.2	99–100 <sup>a</sup>	Normal	Dark	Dark	Dark
Ferric ammonium citrate	17.5	98–115 <sup>a</sup>	Normal	Dark	Dark	Dark
Ferric chloride	20.6	26–67 <sup>a</sup>	Normal	Normal	SI discoloration	Dark
Ferripolyphosphate, gel	18.1	52–60 <sup>b</sup>	Normal	Normal	Normal	Normal
Ferripolyphosphate, aq soln	1.0	83–93 <sup>b</sup>	Normal	Normal	Dark	Dark
Ferripolyphosphate-whey protein	12.5	93–100 <sup>b</sup>	Normal	Dark	Dark	Dark

<sup>a</sup> Data of Fritz et al. (1970)

<sup>b</sup> Data of Jones (1972)

Table 2—Discoloration of iron fortified dehydrated mashed potatoes

Iron compound added	Level of addition (mg Fe/100g)	Color of reconstituted product <sup>a,b</sup>					
		Potato flakes				Potato granules	
		1	2	3	4	5	6
Control	0	N	N	N	N	N	N
Ferric chloride	5	S	D	D	D	D	S
	10	S	D	D	D	D	D
Ferripolyphosphate, gel	5	N	S	N	S	S	N
	10	N	S	D	S	D	N
Ferripolyphosphate, aq soln	5	N	N	N	N	N	N
	10	N	S	N	N	D	N
Ferripolyphosphate- whey protein	5	S	D	D	D	D	D
	10	S	D	D	D	D	D

<sup>a</sup> N = normal color; S = slight discoloration; D = dark.

<sup>b</sup> Commercial products 1–4 (flakes) and 5–6 (granules) were fortified during reconstitution.

from subdivided Idaho Russet Burbank potatoes which had been boiled for 25 min. Coded fortified samples and an unfortified control were examined under fluorescent lighting for color differences immediately and after 30 min (in a boiling water bath) by a panel of at least three judges. Samples were judged subjectively for degree of discoloration in comparison with the control, i.e., normal color, slight discoloration, or dark, and the panel consensus was obtained.

In addition iron compounds were evaluated in a number of commercial dehydrated mashed potato products which represented the commonly used processing methods. Iron compounds were added to mashed potatoes prepared from 15-g portions of each product reconstituted with 85 ml boiling water. Coded fortified products and unfortified controls were examined immediately for color differences by at least three judges as described above.

#### Effect of iron compounds on the storage stability of potato flakes

Iron fortified potato flakes and unfortified controls were produced from Norchip tubers in the pilot plant of the Red River Valley Potato Research Center (East Grand Forks, Minn.) using a modification of the standard process described previously (Sapers et al., 1973). Iron

compounds were added to the mash prior to dehydration at two levels corresponding to 5 and 10 mg Fe per 100g potatoes. On the basis of the initial screening for discoloration, two iron sources were selected for stability testing. These were a ferripolyphosphate solid gel containing 18.1% iron, which was provided by the Milk Properties Laboratory, ERRC, and an aqueous ferripolyphosphate solution containing 1% (w/v) iron, prepared fresh from ferric chloride and Calgon as described by Jones et al. (1972). Flakes were shipped to ERRC, air- and nitrogen-packed in No. 303 and No. 10 cans, and stored at 23° and -18°C for 10 months.

Initial color differences were determined by the same procedure used in the screening studies.

Flavor evaluations were carried out at zero time and after 5 and 10 months storage using a 15-member trained taste panel as described by Sapers et al. (1972). Panelists were asked to rate samples on a five-point scale ranging from five, "same as standard" (control stored at -18°C under N<sub>2</sub>), to one, "extreme off-flavor."

Flake samples were analyzed by gas chromatography after 10 months storage for volatile components associated with lipid oxidation; headspace vapor and volatile concentrate procedures described previously were used (Sapers

et al., 1972). Quantitative results were expressed as the sums of mean peak area ratios (component peak area/internal standard peak area) for major volatile oxidation products as described by Sapers et al. (1973).

## RESULTS & DISCUSSION

### Discoloration in iron fortified mashed potatoes

The addition of iron compounds to both fresh and reconstituted dehydrated mashed potatoes usually resulted in the rapid development of a dark gray-green discoloration (Tables 1 and 2). This defect generally was more pronounced at higher levels of addition. The tendency of a compound to produce discoloration appeared to be more closely related to its biological availability (Fritz et al., 1970; Jones, 1972) than to the oxidation state of iron.

The intensity of color formation varied greatly for the same compound tested in different dehydrated mashed potato products. This variation appeared unrelated to the process (flakes vs. granules) or to the geographic origin of the

Table 3—Stability of iron fortified potato flakes after 10 months in air at 23°C

Flake sample	Mean flavor score			Sum of major volatile oxidation products <sup>a</sup>	
	Storage time (months)			Headspace vapor	Volatile concentrate
	0	5	10		
Control	4.80	4.00	3.33 <sup>b</sup>	0.112	4.22
Ferripolyphosphate, aq soln 5 mg/100g	4.19	2.73 <sup>b</sup>	3.07 <sup>b</sup>	.415	12.60
Ferripolyphosphate, aq soln 10 mg/100g	4.20	3.87 <sup>c</sup>	3.07 <sup>b</sup>	.314	7.43
Ferripolyphosphate, gel 5 mg/100g	2.69 <sup>b</sup>	2.80 <sup>b</sup>	2.94 <sup>b</sup>	.312	11.59
Ferripolyphosphate, gel 10 mg/100g	4.07	3.53 <sup>b</sup>	2.75 <sup>b</sup>	.287	7.42

<sup>a</sup> Mean peak area ratios

<sup>b</sup> Significantly different from hidden standard at 0.01 level

<sup>c</sup> Significantly different from hidden standard at 0.05 level

product; differences in raw material composition and in levels of additives, especially sodium acid pyrophosphate, (SAPP), may have been responsible. This compound is used commercially to inhibit after-cooking darkening in processed potatoes. The addition of 0.03–0.06% SAPP to fresh and reconstituted dehydrated mashed potatoes containing aq. ferripolyphosphate (5–10 mg/100g potato) was found to reduce or eliminate the discoloration in the current study. Unfortunately, any benefits accruing from the use of SAPP in fortified flakes probably would be offset by the poor nutritional availability of the sodium iron pyrophosphate which would be formed (relative biological value of 2–23% according to Fritz et al., 1970).

Of the seven iron compounds screened in fresh and dehydrated mashed potatoes, only the ferripolyphosphate solid gel and aqueous solution showed enough promise to justify further experimentation. When these preparations were used to fortify potato flakes at the Red River Valley Potato Research Center, some discoloration was observed after the addition of the ferripolyphosphates to the mash. Subsequent examinations of reconstituted samples indicated that the fortified mashed potatoes were moderately discolored, the discoloration being more intense at the higher level of addition (10 mg Fe/100g). More discoloration was produced by the aqueous ferripolyphosphate than by the gel, as might be predicted from the higher relative biological value of the former complex.

#### Flavor quality and stability of iron fortified potato flakes

Flavor evaluations conducted at the beginning of the 10-month storage period indicated deficiencies in the flavor quality of all freshly processed iron fortified potato flakes (Table 3). The product fortified with ferripolyphosphate gel (5 mg/100g) received an unusually low flavor score; the flavor of this as well as the other fortified samples was considered to be atypical but not rancid.

During storage, all air-packed samples

and controls stored at 23°C developed off-flavors indicative of oxidative rancidity. After 5 months storage, flavor deterioration was greater in flakes fortified with 5 mg Fe/100g than at the higher level, the two iron compounds giving similar results. All fortified samples were inferior to the control. After 10 months storage, flavor scores of flakes fortified at the 10 mg Fe/100g level declined further, approaching scores received by flakes fortified at the lower level. The unfortified control received a slightly higher flavor score.

Gas chromatographic data show that after 10 months storage in air at 23°C, the fortified flakes contained higher levels of major volatile oxidation products than did the unfortified control. Oxidation product levels were highest in flakes containing 5 mg Fe/100g; similar results were obtained with both iron polyphosphate complexes.

The rather surprising response of potato flakes to fortification level may be due to the proportion of iron in the ferrous and ferric states. This proportion would be determined by such factors as the concentrations of ferripolyphosphate complexes, sodium acid pyrophosphate, hydrogen ion and sulfite. The mechanism of catalysis and extent of oxidation might be expected to vary with the oxidation state of iron (Ingold, 1962). Edmondson et al. (1971) reported that whole milk fortified with iron developed an oxidized flavor with ferrous but not ferric compounds.

#### CONCLUSIONS

FORTIFICATION of dehydrated mashed potatoes with iron at nutritionally significant levels results in after-cooking darkening during processing and reconstitution. The tendency for an iron compound to induce this discoloration is directly related to the relative biological value of the compound.

Iron fortified potato flakes are unstable during processing and storage, developing objectionable off-flavors accompanied by high levels of volatile

components associated with oxidative rancidity.

For these reasons, the use of dehydrated mashed potatoes as a vehicle for iron fortification by the procedures reported herein is not considered to be feasible. An alternative approach to iron fortification, for example, microencapsulation, might warrant further consideration. A suitable encapsulating agent would be required to maintain its structure during product preparation and then undergo biodegradation after ingestion.

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## FLAVOR QUALITY AND STABILITY OF POTATO FLAKES: EFFECTS OF DRYING CONDITIONS, MOISTURE CONTENT AND PACKAGING

### INTRODUCTION

THE SHELF LIFE of dehydrated instant mashed potatoes produced by the flake process is limited by oxidative reactions which result in the development of hay-like off-flavors during storage (Sapers et al., 1972). A recent study of the effects of raw material and processing variables has demonstrated that storage stability is greatly reduced by the use of defective raw material, by the presence of peel in the mashed potatoes during processing, and by cooking and cooling potatoes in excess water such that solubles losses are high. A small decrease in stability resulted from holding peeled raw material prior to further processing. The raw material sugar content and method of subdividing potatoes prior to cooking had little or no effect on storage stability (Sapers et al., 1973).

This research has been extended to other aspects of the potato flake process. Described herein are studies of the effects of drying conditions, product moisture content and packaging on potato flake stability.

### EXPERIMENTAL

#### Raw material and standard process

Potato flakes were prepared from Norchip variety tubers, harvested in North Dakota in September, 1971 and stored at 10°C until required for processing in January and May, 1972. Raw material used in the January experiments had a specific gravity of 1.078–1.092 and contained 0.07% fructose, 0.14–0.17% glucose and 0.24–0.92% sucrose (moisture-free basis); tubers processed in May had a specific gravity of 1.090–1.096 and contained 0.18–0.25% fructose, 0.18–0.30% glucose and 1.24–1.40% sucrose (moisture-free basis).

All flakes were produced at the Red River Valley Potato Research Center pilot plant (E. Grand Forks, Minn.) using modifications of the standard process described previously (Sapers et al., 1973). Sodium bisulfite was added to the mashed potatoes (5 ml of a 1% aq soln per pound) prior to dehydration. Drying was car-

ried out using a single drum drier, 2 ft in diameter and 3 ft in width, having four 3-1/2 in. diameter applicator rolls separated from the drum by 1/8 in. The drier was operated at a steam pressure of 95 psi and a normal drum speed of 2.0–2.4 rpm depending on the consistency of the mash. Finished flakes were packaged in polyethylene-lined fiber drums and shipped to the Eastern Regional Research Center in Philadelphia for repacking, storage and evaluation.

#### Packaging study

Potato flakes prepared by the standard process (drum speed of 2.0 rpm) were air packed in No. 10 cans, completely filled with 600g flakes and also half filled with 300g flakes so that the importance of package headspace volume could be determined. In addition, 600g samples of flakes were air-packed in commercial polyethylene bags (0.0025 in. low density polyethylene) designed for potato flake packaging. The flakes had a bulk density of approximately 0.2 kg/liter as packaged and a density of 0.64 kg/liter after grinding to 20 mesh. All samples were stored at 32°C for 1 yr. Controls were nitrogen packed (less than 2% oxygen) in No. 10 cans and stored at -18°C.

#### Extent of drying and moisture content

Flakes were prepared by the standard process using "slow" drum drying (1.8 rpm) to produce an over-dried product, an intermediate drum speed (2.4 rpm) to produce a normally dried product, and a "high" drum speed (3.5 rpm) to produce an under-dried product. To

differentiate between the effects of drying conditions and the resultant flake moisture content, each of these products were divided into subsets which were humidified by exposure to 65–75% RH at 24°C for 7 hr or cabinet dried at 38°C for 30 min to produce comparable moisture contents. The flakes were then air- and nitrogen-packed in No. 303 and No. 10 cans and stored at -18° and 23°C.

#### Defect accumulation in applicator roll mash

The potentially detrimental effect of defect (fragments of peel, sprouts, rot) accumulation on drum-drier applicator rolls was evaluated in May, 1972 since the incidence of raw material defects is higher at the end of the processing season. Storage potatoes were sorted into two lots, one being relatively free of defects and the other containing numerous defects. Each lot was processed into potato flakes using the standard process with a drum speed of 2.3 rpm. The mash adhering to the applicator rolls during each run was frequently removed and held under ambient conditions until the completion of the run (up to 2 hr). This mash was then processed into flakes. The four products were canned in air and nitrogen and stored at -18° and 23°C.

#### Product evaluation

Initially, potato flake samples were analyzed for moisture, equilibrium relative humidity (ERH), sulfur dioxide and BHA and BHT (Filipic and Ogg, 1960). Analytical data are summarized in Table 1.

Table 1—Moisture and preservatives in potato flakes

Experiment	Moisture content %	Equilibrium rel. humidity %	Preservatives (ppm)		
			SO <sub>2</sub>	BHA	BHT
Packaging study	5.04	16.3	255	23.4	24.0
Under-dried	7.02	33.1	375	20.4	19.5
Under-dried <sup>a</sup>	5.21	18.4	316	20.4	19.5
Normally dried <sup>b</sup>	6.98	36.7	271	23.5	21.5
Normally dried	4.69	16.1	334	23.5	21.5
Normally dried <sup>a</sup>	3.50	8.8	172	23.5	21.5
Over-dried <sup>b</sup>	5.32	20.6	223	20.6	19.6
Over-dried	3.12	6.9	247	20.6	19.6
Good raw material	6.22	31.3	206	21.9	23.8
Good raw material, A.R.M. <sup>c</sup>	6.47	41.2	168	19.8	15.4
Defective raw material	7.10	36.6	168	24.1	23.8
Defective raw material, A.R.M. <sup>c</sup>	6.90	25.6	137	17.9	18.5

<sup>a</sup> Cabinet dried after drum drying

<sup>b</sup> Humidified after drum drying

<sup>c</sup> A.R.M. = applicator roll mash

<sup>1</sup> A Center cooperatively operated by the North Central Region, ARS, USDA; the Minnesota Agricultural Experiment Station; the North Dakota Agricultural Experiment Station; and the Red River Valley Potato Growers Association.

Gas chromatographic (GC) determinations of headspace vapor and volatile concentrate components associated with oxidative reactions and nonenzymatic browning were carried out in duplicate initially and at intervals during storage. GC data were expressed as peak area ratios (component peak area/internal standard peak area) or as sums of peak area ratios for the major oxidation products in sample headspace vapor (4 components) and in volatile concentrates (8 components).

Products were evaluated for flavor quality by a 15-member trained taste panel at the same intervals as the GC determinations. At each session, panelists were asked to rate up to four comparable flake samples from an experiment (including a hidden standard) against a standard, the nitrogen-packed frozen control for the experiment. An 8-point rating scale was used ranging from "much better than standard" (8) to "extreme off-flavor" (1), the flavor rating of the standard being 5 by definition. Results were expressed as mean flavor scores. All procedures and analytical methods employed in the current study have been described previously (Supers et al., 1973).

## RESULTS & DISCUSSION

### Effects of packaging

As with previous studies of potato flake stability, samples in the packaging experiment showed a decline in flavor quality accompanied by increases in volatile oxidation products during storage (Table 2). However, differences between samples were small and not significant.

The extent of oxidative deterioration in air-packed potato flakes apparently is not limited by the volume of air in the package headspace. This is consistent with the work of Buttery et al. (1961) on  $O_2$  absorption, fatty acid composition and off-flavor formation in oxidizing potato granules. If one assumes that a moderate oxidized off-flavor level in flakes is comparable to Buttery's "second detectable difference," then this degree of oxidation will correspond to an unsaturation ratio of approx 1.7 or a 40% loss in linoleic and linolenic acids. Using the fatty acid composition of potato flakes (Schwartz et al., 1968) and Buttery's data on  $O_2$  absorption, one can estimate the  $O_2$  uptake of the flakes equivalent to this loss; 600g of flakes would absorb approx 0.0033 moles of  $O_2$ . The No. 10 can contains about 0.021 moles of  $O_2$ , assuming the density of the flakes to be 1.00. Hence, almost 85% of the original headspace  $O_2$  will remain in a full can containing moderately oxidized potato flakes; the half-full can will retain approx 93% of the headspace  $O_2$ . In this experiment then, there is a large excess of  $O_2$  even in the full can. The importance of package headspace volume would be greater in products having a higher bulk density than the flakes used in this study; i.e., if the bulk density were doubled, approx 40% of the oxygen in the full can

would be consumed with a moderately oxidized product.

The equivalent performance of metal and polyethylene containers in this study is a logical consequence of the insensitivity of the flakes to headspace volume. If the development of an objectionable oxidized off-flavor in potato flakes is not limited by the  $O_2$  content of the container, then the  $O_2$  permeability of the packaging material will not be limiting. Other differences between metal and polyethylene, i.e., permeability to moisture and to volatiles responsible for off-flavor development, apparently were of secondary importance in this experiment.

### Effects of drying and moisture content

Potato flake moisture content and corresponding ERH data from the current experiments and from previously published studies (Supers et al., 1973) were used to estimate the monolayer moisture content by the graphical BET procedure of Salwin (1959). Our value of 5.51% (as is basis) is in good agreement with mono-

layer moisture contents reported by Strolle and Cording (1965) for potato flakes and by Salwin (1959) for dehydrated potato dice.

Stability data for the under-dried, normally dried and over-dried flakes at their original moisture contents are summarized on Table 3. The over-dried flakes contained substantially higher levels of volatile oxidation products than the other samples, initially and after 6 and 12 months' storage. The under-dried flakes were slightly lower in volatile content than the normally dried product. Flavor scores for the under-dried and the normally dried flakes were similar and better than those for over-dried flakes initially and after 1 yr of storage.

These data demonstrate the undesirable consequences of over-drying in commercial practice. They represent the combined effects of two variables: the extent of damage to the flakes during drum drying and the influence of moisture content during storage.

The effect of moisture content on potato flake stability was established inde-

Table 2—Effect of packaging on the stability of potato flakes stored in air at 32°C

Package	Storage time (Months)	Mean flavor score	Sum of major volatile oxidation products	
			Headspace vapor	Volatile concentrate
Fresh flakes	0	4.8	0.062	1.85
Full can	6	3.1 <sup>a</sup>	0.109	3.59
	12	2.8 <sup>a</sup>	0.178	6.49
Half-full can	6	3.7 <sup>a</sup>	0.120	3.07
	12	2.8 <sup>a</sup>	0.177	8.14
Polyethylene bag	6	3.5 <sup>a</sup>	0.134 <sup>b</sup>	3.50
	12	2.5 <sup>a</sup>	0.160	7.03

<sup>a</sup> Significantly different from hidden standard at 0.01 level

<sup>b</sup> 8 months

Table 3—Effect of drying and moisture content on potato flake stability in air at 23°C

Drying	Moisture content %	Storage time (Months)	Mean flavor score	Sum of major volatile oxidation products	
				Headspace vapor	Volatile concentrate
Under	7.02	0	4.9	0.060	1.51
		6	4.3 <sup>a</sup>	0.084	3.27
		12	3.9 <sup>a</sup>	0.116	5.56
Normal	4.69	0	4.7	0.047	1.04
		6	4.0 <sup>b</sup>	0.075	4.27
		12	3.9 <sup>a</sup>	0.130	7.06
Over	3.12	0	4.0 <sup>b</sup>	0.162	3.67
		6	4.2	0.193	9.18
		12	3.3 <sup>a</sup>	0.244	10.60

<sup>a</sup> Significantly different from hidden standard at 0.01 level

<sup>b</sup> Significantly different from hidden standard at 0.05 level

Table 4—Effect of moisture content on the stability of normally dried potato flakes in air at 23°C

Moisture content (%)	Storage time (Months)	Mean flavor score	Sum of major volatile oxidation products	
			Headspace vapor	Volatile concentrate
6.98	0	4.7	0.067	2.87
	6	4.3	0.100	4.29
	12	3.6 <sup>a</sup>	0.156	5.86
4.69	0	4.7	0.047	1.04
	6	4.4	0.075	4.27
	12	3.8 <sup>a</sup>	0.130	7.06
3.50	0	4.7	0.082	2.12
	6	3.7 <sup>a,b</sup>	0.148	6.75
	12	3.7 <sup>a</sup>	0.219	10.85

<sup>a</sup> Significantly different from hidden standard at 0.01 level  
<sup>b</sup> Significantly different from higher moisture samples at 0.01 level

pendently using products which had been drum dried under constant conditions (normal drying at the intermediate drum speed) and then humidified or cabinet dried under mild conditions to the same moisture contents produced by under-drying and over-drying (Tables 1, 4). Levels of volatile oxidation products were higher in the low moisture flakes than in the other samples after 6 and 12 months at 23°C. Flavor scores indicated that the low moisture sample deteriorated as much in 6 months as did the other samples in 12 months; however, flavor differences between samples at 12 months were not significant.

The protective effect of water on the stability of oxygen-sensitive dehydrated foods has been studied extensively (Labuza et al., 1970) and is attributed to the hydrogen bonding of hydroperoxides, deactivation of trace metal catalysts and free radical destruction. In the current research, the instability of the over-dried potato flakes is due in part to their low moisture content.

Another aspect of the problem, the effect of drum drying per se on potato flake stability, was studied using under-dried, normally dried and over-dried flakes which had been equilibrated to approx 5% moisture prior to storage, thereby eliminating moisture content as a variable (Table 5). The over-dried sample received lower flavor scores and contained higher levels of volatile oxidation products than the normally dried and under-dried flakes, initially and after storage. It appears from these data that extensive oxidation can occur during dehydration when the dry potato mash is exposed to air at an elevated temperature for an extended period of time. These conditions may also result in the destruction of naturally occurring antioxidants, i.e., tocopherols and amino acids, which would further destabilize the flakes during storage.

The loss of amino acids would be a consequence of nonenzymatic browning reactions initiated by drum drying and continuing during storage. Levels of 2- and 3-methylbutanal, Strecker degradation products of isoleucine and leucine, respectively, were directly related to the severity of drying and increased during storage (Table 5). The presence of these and other volatile products of nonenzymatic browning reactions may have contributed to the poor flavor of the over-dried flakes (Sapers et al., 1971).

Effect of defect accumulation in applicator roll mash

Sensory and gas chromatographic data summarized in Table 6 indicate that when good quality raw material was used, flakes prepared from conventional mash and applicator roll mash were similar with respect to oxidation during storage. The

Table 5—Effect of extent of drying on the stability of potato flakes equilibrated to constant moisture content and stored in air at 23°C

Extent of drying	Moisture content (%)	Storage time (Months)	Mean flavor score	Sum of major volatile oxidation products		
				Headspace vapor	Volatile concentrate	2- and 3-Methylbutanal <sup>a</sup>
Under	5.21	0	4.9	0.086	2.57	0.063
		6	4.4	0.115	4.56	0.110
		12	4.2 <sup>b</sup>	0.155	6.97	0.137
Normal	4.69	0	4.7	0.047	1.04	0.154
		6	4.0 <sup>b</sup>	0.075	4.27	0.206
		12	3.9 <sup>c</sup>	0.130	7.06	0.227
Over	5.32	0	4.0 <sup>d</sup>	0.168	5.89	0.227
		6	3.8 <sup>c</sup>	0.197	8.68	0.306
		12	3.2 <sup>c,d</sup>	0.233	9.00	0.342

<sup>a</sup> Mean peak area ratio, determined by headspace vapor analysis  
<sup>b</sup> Significantly different from hidden standard at 0.05 level  
<sup>c</sup> Significantly different from hidden standard at 0.01 level  
<sup>d</sup> Significantly different from under-dried and normally dried sample at 0.05 level

Table 6—Effect of applicator roll mash and raw material quality on potato flake stability in air at 23°C

Raw material	Mash	Storage time (Months)	Mean flavor score	Sum of major volatile oxidation products		
				Headspace vapor	Volatile concentrate	2- and 3-Methylbutanal <sup>b</sup>
Good	Conv.	0	5.0	0.047	1.69	0.218
		6	3.9 <sup>c</sup>	0.075	4.79	0.256
		12	3.6 <sup>c</sup>	0.128	6.80	0.312
Good	A.R. <sup>a</sup>	0	4.6	0.062	1.38	0.466
		6	3.3 <sup>c,d</sup>	0.122	4.97	0.519
		12	3.2 <sup>c</sup>	0.140	6.92	0.577
Defective	Conv.	0	4.5	0.049	1.10	0.502
		6	3.9 <sup>c</sup>	0.097	3.36	0.610
		12	2.8 <sup>c</sup>	0.096	6.07	0.650
Defective	A.R.	0	3.7 <sup>c</sup>	0.048	2.09	0.702
		6	3.2 <sup>c,d</sup>	0.126	6.37	0.727
		12	2.5 <sup>c</sup>	0.131	8.96	0.810

<sup>a</sup> A. R. = applicator roll  
<sup>b</sup> Mean peak area ratio, determined by headspace vapor analysis  
<sup>c</sup> Significantly different from hidden standard at 0.01 level  
<sup>d</sup> Significantly different from conv. mash at 0.05 level

slightly lower flavor scores received by the applicator roll mash flakes initially and during storage may be due to nonenzymatic browning reactions. Levels of 2- and 3-methylbutanal were higher in this product than in the conventional mash flakes.

Flakes processed from defective raw material were poorer in flavor quality initially and less stable during storage than were the flakes made from good raw material. The applicator roll mash flakes contained higher levels of volatile oxidation products and 2- and 3-methylbutanal than did the conventional mash flakes. The very low flavor scores received by both products after 12 months' storage probably reflect the combination of oxidized and browning off-flavors.

It would appear from these results that the drum-drier applicator rolls are a potential source of off flavors and product instability, and should be cleaned frequently, especially when the raw material has a high incidence of defects. Willard (1968) has proposed that the applicator rolls be cooled to alleviate this problem. Defects should be removed from the

product prior to dehydration by careful inspection and trimming of the raw tubers.

### CONCLUSIONS

WE CONCLUDE that package headspace volume and the choice of polyethylene or metal as the packaging material do not affect the storage stability of air-packed potato flakes having a low bulk density.

Over-drying decreases the stability of air-packed potato flakes; this is a consequence of the sensitivity of dehydrated foods to oxidation at low water activities and to the damage done to the product during dehydration.

Potato flake flavor quality and stability may be affected adversely by the build-up of raw material defects in the mash adhering to drum drier applicator rolls.

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## PREPARATION AND STORAGE PROPERTIES OF DRUM DRIED WHITE YAM (*Dioscorea rotundata* Poir) FLAKES

### INTRODUCTION

ONE OF THE problems aggravating food shortages in developing countries is a lack of adequate inexpensive preservation methods for local foods to ensure their supply throughout the year. This is especially true for tropical yams.

Yams, the edible tubers of various species of the genus *Dioscorea*, are important items in the diet of many people in tropical countries, especially West Africa (Coursey, 1965; Gusten, 1968). Yams are widely cultivated in many parts of West Africa, especially Nigeria, which produces more than half of the world's annual crop (Ingram and Barton, 1962). The unique role of yam in the nutrition, economy and social and religious festivities of Nigeria has been described in detail (Nicol, 1952; Johnston, 1958; Coursey, 1967; Gusten, 1968).

There are many species of yam in cultivation, and these can be distinguished by a number of characteristics such as the size and shape of the leaves and tubers and characteristics of the starch granules (Rasper and Coursey, 1967). Most species of yam are edible after cooking; some also are used in traditional medicine and as precursors of certain pharmaceutical alkaloids and steroids (Bevan and Hirst, 1958).

Yam is not only a good source of starch but consumed in quantity is capable of providing the greater part of the dietary requirement of vitamin C. Yam peelings have been used in local communities to raise sheep and goats which supplement the protein supply, but the high cost of production precludes its widespread use as livestock feed in much of West Africa.

Yam is a high moisture bulky crop. The nature and extent of the losses involved in the marketing and storage of yam by the West African farmer have been described by Coursey (1965), Anthonio (1967) and Are (1971).

In most areas where yam is a staple of diet, yam preservation and processing are done in the traditional way which results in considerable food loss. Attempts to preserve raw yam by using sprout inhibitors, controlled atmosphere, or low temperature storage have not been successful (Coursey, 1961). This lack of suit-

able preservation methods accentuates the seasonal fluctuation in raw yam prices. Yam flour is the only locally processed form of yam in much of West Africa. It is obtained by sun drying peeled yam pieces and milling the hard pieces. The yam flour thus produced is reconstituted with hot water before eating. This product has limited acceptability and distribution among yam consumers because of its dark color and poor texture when reconstituted.

The most important food yam in West Africa is *Dioscorea rotundata* Poir. It is suitable for mashing after cooking, frying as slices in vegetable oil, or as a major constituent of soups and other food mixtures. Mashed yam (Iyan-Yoruba, or fufu as it is called in Ghana) is by far the most important food prepared from yam in West Africa.

Besides the recent work of Rodriguez-Sosa and Gonzalez, (1972a, b) little has been published on the dehydration of yam (*Dioscorea* spp.) and its properties. However, there is substantial information on the production of potato flakes (Cording and Willard, 1956, 1957; Drazga and Eskew, 1962; Feustel et al., 1964) and sweet potato flakes (*Ipomoea batatas*—incorrectly called 'yam' in the U.S.) (Spadaro and Patton, 1961; Deobaid et al., 1962; Hoover and Harmon, 1967; Spadaro et al., 1967), the technology of which can be applied to yam.

### MATERIALS & METHODS

#### Source of yam and analyses

The white yam, *Dioscorea rotundata* Poir, was imported from Columbia. This yam is of the same variety as grown in West Africa. Selected yams were washed, peeled and the edible portion analyzed for moisture, starch, protein and ether extract by standard methods (AOAC, 1970). Total reducing sugars were determined by the method of Ross (1959) using 2,4-dinitrophenol. Specific gravity of the raw tubers was measured by weighing in air and in water. Blue value index of the raw, cooked and dehydrated yam flakes was obtained by the method of Mullins et al. (1955). Total and reduced ascorbic acid in the raw, cooked and dehydrated yam were determined by the methods of Roe and Osterling (1944) and Mapson (1942). Color of freshly dried and stored yam flakes was determined objectively with the Hunterlab Color and Color Difference Meter (Hunterlab Associates).

#### Preparation and drying

The peeled yam was sliced into approximately 0.5 inch thick pieces, dipped in 1.5% sodium bisulfite solution for 1 min to prevent browning before and after cooking, drained and steam cooked in a pressure cooker for 30 min at 5 psi steam pressure. Cooking under 30 min led to darkening of the yam pieces before mashing despite the bisulfite dip, and the undercooked yam produced a grainy mash.

The cooked yam was diluted with water as necessary to produce a mash of 20% solids to facilitate subsequent flow through the nip between drying drums. The dilution water contained small quantities of additives commonly employed to enhance the quality of dehydrated potato flakes (Feustel et al., 1964), and the concentration of these additives in the final mash amounted to 11 ppm SO<sub>2</sub>, 5.2 ppm BHT, 0.026% emulsifier (MYVEROL 1800, Eastman Kodak), and 0.026% nonfat dry milk. The cooked yams and diluting solution were gently mashed to minimize starch granule rupture in a Hobart mixer (Model 200) fitted with a wire whip at speed 2 for 4 min. Batch size was 10 lb.

The mashed yam was dehydrated on a Blaw-Knox laboratory atmospheric double drum dryer with two 6-in. diameter × 7 5/8 in. length drums. Drum speeds were varied between 2–4 rpm with a clearance between the drums of approximately 0.01 in. The drums were heated by steam at a pressure of 80 psi. The mashed yam was force fed through the nip between the drums with mild pressure from a rubber spatula.

#### Storage conditions

Representative samples of yam flakes dried to different moisture levels at different drum speeds were stored in air tight bottles up to 90 days within incubators at 21.1°C, 29.4°C and 37.8°C. Samples of the stored flakes were evaluated at 30-day intervals for color, flavor and ascorbic acid changes.

#### Taste panel evaluations

A 15 member panel group consisted of African students at Cornell University who were familiar with cooked yam and represented most African countries that are likely to be potential markets for a dehydrated yam product. Flakes were reconstituted by mixing 1 part with 3 parts of hot water. This ratio gave acceptable texture compared to conventionally prepared African mashed yam.

Flavor preference between cooked-mashed yam and reconstituted freshly dried yam flakes was measured by a triangle test (Kramer and Twigg, 1962). Flavor changes in reconstituted stored flakes were assessed by a rank order test using a 9-point hedonic scale (Amerine et al., 1965). Taste panel members also were requested to comment on color, texture and overall acceptability.

## RESULTS &amp; DISCUSSION

## Composition of yam

The composition of the edible portion of the white yam, *D. rotundata* Poir, used to produce the yam flakes is shown in Table 1. The data represent ranges from three determinations for each constituent on the yam as received at different intervals. Variations in the composition of the yam were remarkably small considering that the yam batches probably were not from a single farm and may have encountered variation in time of planting, climate, harvesting and post-harvest handling. The specific gravity and total solids of the yam are high. The high starch and low total reducing sugar content of the yam indicate its suitability for dehydration, as in the case of potato. These criteria, however, may be insufficient in themselves to predict yam dehydration properties since yams may further differ in their starch granule and cooking characteristics (Rasper and Coursey, 1967). The ascorbic acid content of the raw tuber was low as expected with most root crops. Cooking prior to dehydration further decreased ascorbic acid levels by approximately a third.

## Dehydration of yam

Typical data on the preparation of yam flakes are shown in Table 2. Discoloration of peeled yam prior to cooking could be minimized by keeping the yam under water. The sulfite dip, as it is prac-

ticed with potato flake processing, prevented the discoloration of the yam before and after cooking. This suggests that the mechanism of discoloration in yam is identical to that in potato and can be controlled by the same treatments (Smith, 1957).

The yield of yam flake solids based on peeled cooked tuber approached 100% recovery. The high peeling loss of approximately 30% included nonedible portions of the yams as well as peels. This loss can probably be reduced somewhat in commercial practice. The moisture content of the yam flakes increased gradually from 3.5–7.8% as the drum speed was changed from 2–4 rpm. Beyond these drum speeds, the moisture content of the yam flakes exceeded the range shown to be necessary for good storage stability of potato flakes (Salwin, 1963; Strolle and Cording, 1965).

Table 3 shows the changes in the blue value index of the raw, cooked, mashed and reconstituted yam flakes. These results indicate that there was practically no free starch in the raw yam tuber and this increased only slightly on cooking. Mashing, however, produced a great increase in the blue value index which did not further change during drying. The high blue value index of the yam flakes correlated with the increased pastiness of the reconstituted flakes compared to the cooked yam. A high blue value index for mashed yam and the reconstituted flakes,

however, does not have the same significance as in the case of potato because people who consume mashed yam are used to moderate pastiness and prefer it to mealiness.

## Storage properties

Color changes in the stored yam flakes are expressed as total color differences ( $\Delta E$ ). These were calculated from the differences between the tristimulus values of L, a and b, for the representative stored samples and the corresponding values from samples of freshly dried yam flakes of the same moisture content. Color change data, measured at 30-day intervals, are shown in Table 4. Statistical analyses of these color difference data indicate that time, temperature and level of moisture in the yam flakes all had statistically significant ( $P < 0.01$ ) effects upon the color changes calculated. The data also indicate that yam flakes containing 3.5 and 5.8% levels of moisture stored at either 21.1°C or 29.4°C had color changes which were of lesser magnitude than the flakes containing 7.8% moisture stored at 37.8°C. The color differences, however, were not of a magnitude that could be readily discerned visually and this would indicate that these yam flakes would be stable in color in the tropics (up to 37.8°C) for at least 90 days. The stability of this product to color change agrees with published observations by Eastmond et al. (1951).

Results of organoleptic acceptability tests (by triangle test method) indicated that tasters actually preferred ( $P < 0.05$ ) the reconstituted fresh yam flakes to the conventionally mashed yam. Smoother texture frequently was cited for the preference. The ratings from the rank order test on the hedonic scale were converted to numerical scores as shown in Table 5. Average flavor scores from 15 tasters are given and represent rankings in relation to a flavor score of 9 assigned to freshly dehydrated flakes of 3.5% moisture. All stored samples scored lower than the freshly dried control. Analysis of variance revealed that time, temperature and moisture content of the flakes all had statistically significant ( $P < 0.01$ ) effects upon flavor. Generally the flavor scores decreased with increased time and temperature but increased moisture level

Table 1—Composition of the edible portion of *D. rotundata* Poir

Determination	Range	
Moisture	75.9–76.3%	
Total solids	23.7–24.1%	
Starch	21.4–22.5%	
Total reducing sugars	0.9–1.0%	
Protein (N X 6.25)	1.0–1.1%	
Ether extract	0.1–0.2%	
Specific gravity	1.086–1.095	
	Raw yam	Cooked yam
Total ascorbic acid (mg/100g)	6.8–7.2	4.4–4.6
Reduced ascorbic acid (mg/100g)	6.6	4.2

Table 2—Operational data on preparation of yam flakes

Weight of yam	14.5 lb
Peeling loss	4.3 lb
Weight of cooked yam	10.3 lb
Time of cooking	30 min
Pressure of steam cooker	5 psi
Weight of mashed yam (diluted to 20% solids)	11.8 lb
Drum speed	2–4 rpm
Drum steam pressure	80 psi
Clearance between the drums	0.01 in.
Weight of yam flakes (96.5% solids)	2.3 lb

Table 3—Changes in the blue value index of yam through processing

Form of yam	% Total solids	BVI <sup>a</sup>
Raw yam tuber	24	5
Cooked yam	23	16
Mashed yam	20	240
Yam flakes	96.5	235

<sup>a</sup> Based on yam total solids of 24%

showed a less consistent pattern, suggesting minimum stability at the moisture level of 5.8%. Additional drying trials would be necessary to determine if this latter observation is valid. These flavor changes, however, were not of a magnitude to cause tasters to reject any of the stored samples as unacceptable, and an additional evaluation by three randomly chosen members of the panel revealed that 3.5% moisture flakes stored 120 days at 37.8°C remained quite acceptable.

Besides flavor, other comments by the tasters varied according to their native country and previous experience. Some tasters liked the smooth texture of the reconstituted mash but wanted the mash to be more pasty. Tasters from other areas preferred greater mealiness. These personal preferences, and the fact that the mashed yam was served without vegetables, as is a custom in parts of West Africa, undoubtedly influenced ratings given the product.

Ascorbic acid losses resulting from cooking raw yam (Table 1) were of the order of 33%. Subsequent mashing and drying further decreased total and reduced ascorbic acid by approximately 45% to average levels of 2.5 and 2.2 mg/100g of freshly dried reconstituted flakes. Subsequent storage stability of total ascorbic acid is seen in Table 6. Ascorbic acid values in this table represent an average of three determinations

on each stored sample. There were only very slight changes in total ascorbic acid (and reduced ascorbic acid) contents of the stored flakes up to 90 days regardless of the temperatures of storage or moisture contents of the flakes. This indicates that ascorbic acid supplementation of cooked yam prior to drying would be feasible in terms of stability of the residual vitamin in the stored flakes. Supplementation with ascorbic acid, however, adds to cost and may not be justified in regions where yams are traditionally eaten with other vegetables containing this vitamin.

CONCLUSIONS

THIS STUDY indicates that the white yam *Dioscorea rotundata* Poir can be easily dehydrated by drum drying to yield quality flakes that should be readily accepted by West African consumers. Storage stability of these flakes for at least 90 days at temperatures up to 37.8°C were generally good with respect to color, flavor and other attributes. Ascorbic acid losses were substantial during cooking and drying but residual ascorbic acid was stable during subsequent storage of yam flakes. No attempt was made to further improve storage stability of dehydrated flakes but it is expected that studies on optimum levels of additives commonly employed in the dehy-

dration of potato would produce positive results.

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Table 4—Color changes (ΔE) of stored yam flakes<sup>a</sup>

Flake moisture	Stored 30 days			Stored 60 days			Stored 90 days		
	21.1°C	29.4°C	37.8°C	21.1°C	29.4°C	37.8°C	21.1°C	29.4°C	37.8°C
3.5%	1.6	1.2	1.5	1.2	1.2	2.3	1.6	1.5	2.3
5.8%	2.4	2.3	2.0	2.4	2.6	2.5	2.2	2.1	2.2
7.8%	2.8	2.8	2.8	2.8	2.8	3.0	3.2	3.1	3.3

<sup>a</sup> Freshly dried yam flakes of identical moisture contents were used as standards.

Table 5—Flavor scores of reconstituted freshly dried and stored yam flakes<sup>a</sup>

Flake moisture	Stored 30 days			Stored 60 days			Stored 90 days		
	21.1°C	29.4°C	37.8°C	21.1°C	29.4°C	37.8°C	21.1°C	29.4°C	37.8°C
3.5%	6.8	7.4	7.4	6.8	6.8	7.2	7.7	5.8	5.2
5.8%	7.4	7.0	—	6.8	5.4	—	6.5	4.5	—
7.8%	8.5	8.5	8.3	6.5	6.8	7.0	7.9	7.6	6.3

<sup>a</sup> Freshly dehydrated yam flakes were assigned a flavor score of 9.

Table 6—Total ascorbic acid content of stored yam flakes<sup>a</sup>

Flake moisture	Stored 30 days			Stored 60 days			Stored 90 days		
	21.1°C	29.4°C	37.8°C	21.1°C	29.4°C	37.8°C	21.1°C	29.4°C	37.8°C
3.5%	2.6	2.5	2.4	2.5	2.5	2.4	2.5	2.5	2.4
5.8%	2.5	2.5	2.4	2.5	2.5	2.3	2.5	2.4	2.4
7.8%	2.5	2.5	2.4	2.4	2.4	2.3	2.4	2.3	2.3

<sup>a</sup> In mg/100g reconstituted flakes

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## COLOR OF PROCESSED SWEET POTATOES: EFFECTS OF CAN TYPE

### INTRODUCTION

THE TIN COATING of cans has been recognized to have a beneficial effect on the color of several products, including beans and asparagus (Hernandez and Vosti, 1963; Hotchner and Kamm, 1967; Van Buren and Downing, 1969). Generally, the product of tin-coated cans has a brighter appearance with less darkening than that processed in enamel-lined cans. Until recently, tin-coated containers have been widely used in the processing of sweet potatoes. A change to enameled containers has been made in the past few years due to the troubles encountered with can corrosion (de-tinning) in sweet potato packs in certain areas. The present study was initiated to determine the effect of type of can on color of the processed product.

### EXPERIMENTAL

'NEMAGOLD' sweet potatoes were grown on the Vegetable Research Farm of the University of Maryland located near Salisbury, Md. and processed in the laboratories of the Dept. of Horticulture at College Park. Roots 1-1-3/4 in. diam were peeled in 10% boiling lye for ca 3 min, washed, trimmed, and 300g (fresh wt) packed into No. 303 cans. A 25% sucrose syrup was added to the syrup pack and processed 35 min at 116°C. The vacuum pack was evacuated and processed 45 min at 116°C.

Six types of No. 303 cans with the following specifications were used:

Can designation	Interior coating
C-1	Plain tin, electrolytic plate (Type L), 1 lb tin
C-2	Plain tin, hot-dip plate (Type L), 1 1/2 lb tin
C-3	"C" enamel (an oleoresinous type with suspended zinc oxide pigment) on 1/2 lb tin
C-4	"C" enamel on 1/4 lb tin with high-tin (30%) solder filleted side seams
C-5	"F" enamel (an oleoresinous type) on 3/4 lb tin
C-6	"C" enamel on 1/4 lb tin

All six types were used in the main experiment. Three other lots were processed at different times during the season with both freshly harvested and stored sweet potatoes and packed in electrolytic plate (C-1) and enameled (C-6) cans.

Visual evaluation of color was made by three experienced judges on unidentified samples. The rating was made on attractiveness

or brightness of color rather than on apparent amounts of the carotenoid pigment present. Objective color evaluations were made with the Hunter Color and Color Difference Meter standardized on a tile with values of  $L = 58.6$ ,  $a_L = 29.4$  and  $b_L = 32.0$ . Surface areas of the roots were presented to a 1 in. diam opening on the instrument. Five roots from each can were read and the arithmetic average used as the value of the can contents. Tin content of the different packs was determined by atomic absorption flame photometry using a Perkin-Elmer Model 303 instrument. Means presented tabularly are from two or more cans of a treatment.

### RESULTS & DISCUSSION

VISUAL SCORES and Hunter values obtained 12 months after processing showed a definite difference between the product in the plain and enameled cans, with that in the plain cans more attractive and brighter in appearance and showing higher Hunter L values (Table 1). Significant visual differences were not detected between the electrolytic plate and the hot-dip cans (C-1 and C-2) nor among the several types of enameled cans. Certain Hunter values within the two types of cans were significantly different but the principal difference was between the plain and enameled cans. The high tin fillet of the C-4 can resulted in a higher L value of the product from the syrup pack but not of the vacuum pack. The color

difference  $\Delta_E$  (Hunter, 1958) calculated as:  $\Delta_E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$  between the two plain-type cans (C-1 and C-2) and the four types of enameled cans (C-3, C-4, C-5 and C-6) was 4.08 for the syrup pack and 4.69 for the vacuum pack.

In the three lots with only two can types, all lots showed differences both visually and in the Hunter values between the two can types, with the product from the plain can being brighter, clearer, more attractive in color and with higher Hunter values (Table 2). Vacuum packs normally show more darkening and discoloration than syrup packs of sweet potatoes. This is evident in the present tests but the beneficial effect of the plain can was similar with the two packs.

Hunter readings were made at the time of opening the cans and after exposure of the roots to air for 1 hr, with comparative readings on the same roots at the same location. The changes in Hunter values during the exposure period are given in Table 3. The product from the plain cans showed much less darkening upon exposure than from the enameled cans. The greater visual differences in brightness between the two types of cans after exposure were quite apparent. The results (Table 3) show higher tin values for the electrolytic plate than for the hot-dip can, and higher values for the vacuum than for the syrup pack. The higher con-

Table 1—Visual scores and Hunter color meter values of sweet potatoes packed in different can types

Can type	Visual score of appearance <sup>b</sup>		Hunter CDM value <sup>a</sup>					
	Syrup pack	Vacuum pack	Syrup pack			Vacuum pack		
			L	a	b	L	a	b
C-1 (Plain)	9.4	8.1	55.1	17.1	25.7	53.8	15.7	24.5
C-2 (Plain)	9.1	8.3	54.4	16.8	25.7	54.5	15.6	25.4
C-3 (Enamel)	6.8	5.3	52.8	15.8	23.7	50.6	14.0	22.0
C-4 (Enamel) <sup>c</sup>	7.1	6.1	53.8	16.3	26.8	48.7	11.7	23.2
C-5 (Enamel)	6.8	5.3	50.9	15.0	24.8	51.8	14.0	25.1
C-6 (Enamel)	7.0	6.1	50.3	14.3	24.7	49.0	12.5	23.3
L.S.D. (5% level)	1.3	1.3	2.0	2.0	1.5	2.0	2.0	1.5

<sup>a</sup> Values are means of four cans.

<sup>b</sup> Scored on a 1-10 basis with 10 most desirable color, and 6 indicating borderline acceptability.

<sup>c</sup> C-4 had a high-tin solder fillet in the side seam.

Table 2—Comparison of sweet potato packs from "plain" and "enameled" cans

Can type	Visual score of appearance		Hunter CDM value <sup>a</sup>					
	Syrup pack	Vacuum pack	Syrup pack			Vacuum pack		
			L	a	b	L	a	b
Lot 2								
C-1 (Plain)	8.7	8.2	55.8	18.7	29.6	55.5	17.5	29.2
C-6 (Enamel)	8.5	7.8	50.8	14.5	25.2	50.0	14.1	24.6
Lot 3								
C-1 (Plain)	8.9	8.5	56.9	18.1	29.7	56.5	16.1	28.7
C-6 (Enamel)	8.6	8.1	53.5	14.4	26.9	52.4	13.3	25.4
Lot 4								
C-1 (Plain)	8.6		56.9	19.6	29.9			
C-6 (Enamel)	8.2		53.0	15.5	26.4			

<sup>a</sup> Visual scores were made 1 month after processing. Hunter readings were made 1 yr after processing.

Table 3—Effect of container type on tin content and change in Hunter CDM values after exposure of processed sweet potatoes

Can type	Tin (ppm of can contents)		Change in CDM values <sup>a</sup>	
	Syrup pack	Vacuum pack	Syrup pack	Vacuum pack
C-1 (Plain electrolytic)	78	223	1.66	1.44
C-2 (Plain, hot cip)	53	81	1.34	0.85
C-3 (Enamel)	3	2	4.70	2.86
C-4 (Enamel, high tin fillet)	13	9	3.50	2.45
C-5 (Enamel)	3	4	3.45	4.06
C-6 (Enamel)	4	2	2.36	2.80

<sup>a</sup> Calculated as  $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$

tent of the vacuum pack may be partly a result of the dilution effect of the syrup, but it was also noted that can surfaces in contact with the contents of a vacuum pack were severely de-tinned. The enamel-lined cans showed slight tin content. The high-tin fillet can type (C-4) had a much lower tin content than the tin-coated cans. The cultivar 'Nemagold' used in these tests has been found to be relatively less corrosive and therefore has a lower tin content of the processed product than certain other cultivars (Smittle and Scott, 1969).

Darkening or discoloration of processed sweet potatoes is a result of action of the polyphenolase complex (Scott et al., 1944), with the degree affected by cultivar, method and severity of peeling, exposure to air after peeling and other factors. The reaction occurs in two phases: first the oxidation of hydroxyl groups of orthodihydroxyl phenolic compounds to the corresponding quinone; and second, the reaction of the quinones to form dark-colored substances. The first phase is controlled by the polyphenolase

system, the second phase may proceed nonenzymatically (Smittle and Scott, 1969). Since both phases require oxygen, exposure to air either before or after processing may increase the development of discoloration.

Hernandez and Vosti (1963) have shown with asparagus that in the presence of stannous salts, yellow colored pigments are formed while in the presence of ferrous salts, the pigments formed are dark in color. The reactions in the sweet potato seem to be similar since we noted that the addition of stannous chloride to a dark extract from the discolored product of enameled cans resulted in a bright yellow color of the extract. It is assumed that the tin removed by processed sweet potatoes from the can surface effectively competes with the iron present in the product. This assumption is supported by the significant (0.05) negative correlation coefficient (-0.63) between the tin content of the product and its tendency to darken upon exposure to air. The effects of tin and iron on color appear to be reversible since the color of blended

sweet potatoes can be changed by successive additions of stannous and ferric chloride. Additionally, we have observed darkening of the product in severely corroded cans after prolonged storage, presumably caused by exposure of the product to the iron underlying the tin coating. It is postulated, therefore, that the presence of tin derived from the can coating does not prevent the reactions originated by the polyphenolase system but that the complex formed is of a color that is not deleterious to the appearance of the processed product. Processors have noted that sweet potato packs "bleach out" upon holding, and have relied on this effect to produce acceptable appearing packs even when considerable discoloration has occurred during preparation. The present tests indicate that such a bleaching effect will not be obtained in enamel-lined cans. It was also found that the amount of tin present in the "high-tin fillet" can was insufficient to appreciably affect the color of the product.

When enameled cans are used with sweet potatoes, discoloration during preparation must be avoided to insure a bright colored product. Varietal selection (Scott and Kattan, 1957), preheating (Scott et al., 1945), and rapid handling will aid in this respect. If discoloration cannot be prevented, then additives to substitute for the action of the tin could give the desired effects. Results of such tests have been submitted for publication (Twigg et al., 1974).

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## COLOR OF PROCESSED SWEET POTATOES: EFFECT OF ADDITIVES

### INTRODUCTION

THE IMPROVED COLOR of processed sweet potatoes packed in tin-coated cans as compared with those packed in enamel-lined cans has been shown (Scott et al., 1974). With the present trend toward the use of enamel-lined cans to avoid corrosion problems, the difficulty of obtaining a desirably colored and attractive produce has increased. Discoloration during the processing procedure has been shown to be caused by the polyphenolase system in the sweet potato (Scott et al., 1974). The degree of discoloration is affected by variety (Scott and Kattan, 1957), processing procedures (Scott et al., 1945) and possibly other factors. Van Buren and Downing (1969) reported stannous chloride to be effective in improving color of wax beans while EDTA and citrate were only slightly effective. Sistrunk (1971) reported treatment with citric acid improved the color of processed sweet potatoes. The present work was initiated to determine the effectiveness of chemical additives to prevent discoloration or improve the color of the processed product.

### MATERIALS & METHODS

SWEET POTATOES were grown at the University of Maryland's Vegetable Research Farm, Salisbury, Md. 'Nemagold', the principal processing cultivar of the Mid-Atlantic area which is moderately susceptible to discoloration, was used for all tests. Preheated lots were held in 57°C water for 20 min prior to peeling. Potatoes were peeled in 10% boiling lye for ca 3 min, washed, trimmed and 300g packed in No. 303 cans. Hot 25% sucrose syrup was added to the syrup packs before cooking at 116°C for 35 min. Vacuum packs were evacuated, closed, and cooked for 45 min at 116°C.

In the vacuum packs, the peeled potatoes were dipped into the indicated solution for 30 sec, drained and packed into the cans. In the syrup packs, a given quantity of the solution was added to the can just before filling with syrup.

Two lots of sweet potatoes were used, the first harvested on Oct. 20 and processed on Oct. 28; the second lot was harvested on Oct. 30 and processed Nov. 4. Additives used with Lot 1 were the sodium salt of ethylenediaminetetraacetic acid (EDTA), citric acid and ascorbic acid as 1.5 and 3.0% solutions and stannous chloride (SnCl<sub>2</sub>) as 0.5 and 1.0% solutions. 10 ml of solution was added to each can. In Lot 2, treatments consisted of variable amounts of EDTA, citric acid and sodium bisulphite. Tin-coated and enamel-lined cans without additives

were included as controls with each lot. Half of each lot was preheated, the remainder were peeled without preheating. Eight cans of each treatment in each lot were processed.

Color was evaluated visually by a panel using unidentified samples, and objectively by the use of the Hunter Color and Color Difference Meter standardized on a plate with values L 58.6, a<sub>L</sub> 29.4 and b<sub>L</sub> 32.0. Hunter readings were made at time of opening cans and again on the same samples after exposure to air for one hour in the laboratory. Panel scores were on basis of 1-10 with higher scores indicating more desirable color. Generally, the principal factor scored was the presence of the darkening compounds which caused the product to be dark or grayish in color or the absence of darkening resulting in the normal clear orange of the carotenoid pigments. In some instances the darkening appeared as streaks on the surface of the potatoes.

### RESULTS & DISCUSSION

VISUAL SCORES were made after 1, 3 and 20 months (Table 1). The EDTA, SnCl<sub>2</sub> and citric acid treatments were quite effective in improving the color of the product in the enamel-lined cans. At the first two examination dates, the ascorbic acid treatment was effective, but this effect was lost after extended hold-

ing. The combination of the lower concentrations of ascorbic and citric acids was not effective. The higher concentration of the additive did not result in consistently higher scores. The means presented in Table 1 are means of syrup and vacuum packs and preheated and nonpreheated roots, since the treatment × pack and the treatment × preheating interactions were not significant at any date.

Hunter L, a and b values of the sweet potatoes obtained after 10 months storage were higher for the additive treatments than for those in the enamel-lined cans, corroborating the visual observation of lighter and brighter appearance of the treated potatoes. Differences were greatest in L and a values. Color differences between the several treatments and the enamel-lined control calculated as  $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$  (Hunter, 1958) at time of opening the cans and 1 hr later are given in Table 2. Since  $\Delta E$  was calculated from means, statistical treatment was not appropriate. The EDTA, SnCl<sub>2</sub> and citric acid treatments were effective in improving the color of both syrup and vacuum packs as indicated by the color difference from the enamel-lined control

Table 1—Effect of additives on visual color score of processed sweet potatoes

Additive treatment <sup>c</sup>	Visual color score <sup>a</sup>		
	After 1 mo storage	After <sup>b</sup> 3 mo storage	After 20 mo storage
1½% EDTA	9.3	9.2	8.7
3% EDTA	9.4	9.5	8.6
½% SnCl <sub>2</sub>	9.0	8.9	8.6
1% SnCl <sub>2</sub>	9.3	9.2	8.5
1½% Citric acid	8.6	9.1	8.2
3% Citric acid	8.9	9.1	8.5
1½% Ascorbic acid	9.0	9.0	8.3
3% Ascorbic acid	8.8	9.3	8.3
1½% Ascorbic acid + 1½% Citric acid	8.8	9.0	7.5
Plain can (control)	8.6	9.4	9.0
Enameled can (control)	8.4	8.6	7.6
L.S.D. (5%)	0.4	0.5	0.6

<sup>a</sup> Scores are means of four cans.

<sup>b</sup> Pre heated lot only scored on this date

<sup>c</sup> All additive treatments packed in enamel-lined cans

pack. The ascorbic acid and ascorbic acid-citric acid combination treatments were much less effective, particularly with the vacuum pack. In general, differences among treatments were greater after exposure for one hour than at time of opening. The rate of darkening of the several treatments is indicated in Table 3 which gives the color difference calculated between Hunter values at time of opening and after 1 hr exposure to air. The EDTA and SnCl<sub>2</sub> treatments had about the same effect as the plain can in preventing darkening upon exposure. The citric and ascorbic acid treatments were

much less effective and the combination citric-ascorbic treatment was ineffective.

The second lot employed variable quantities of a 3% solution EDTA and citric acid, and a 1% solution sodium bisulphite added to the syrup pack. Visual scores made 3 mo after processing (Table 4) indicate that EDTA was quite effective in improving appearance of the processed product, even at the lower concentration. Citric acid and sodium bisulphite were not effective except at the higher levels. The liquor in the EDTA treatments was clearer, more yellow and lacked the greenish appearance of the

control lot packed in enamel-lined cans.

The color difference values obtained 1 yr after processing are in general accord with the visual ratings, showing the EDTA treatment to be effective at all levels but the citric acid and bisulphite treatment effective only at the higher levels. The bisulphite treatments are also ineffective in preventing darkening upon exposure to air after opening the cans.

The additives used in these tests improved the appearance of the processed product, presumably through an inhibitory effect on the nonenzymatic reactions of the quinones rather than by the prevention of the initial quinone formation by the phenolase system. Darkening which occurred upon exposure of the processed product is assumed to result from nonenzymatic reaction of the quinones produced during the initial phenolase activity. Lack of oxygen in the closed cans largely prevented this stage of the reaction from taking place during storage.

EDTA was found to be most effective of the additives used, functioning at low concentrations, and particularly effective in preventing the darkening of the product upon exposure to air. The action of EDTA may be due to the chelation of metallic ions which are responsible for the formation of the dark-colored quinone complex. Since the addition of 10 ml of 3% solution per can resulted in only a minor pH change (from 5.3 to 4.9), the action of citric acid may also have been as a chelating agent. Sistrunk (1971) reported improved color or attractiveness of processed sweet potatoes with decreasing pH from 8 to 3 effected by treatments with citrate buffer. Ascorbic acid may have functioned by preventing the formation of quinone condensation products through a reduction reaction. The addition of tin in the form of stannous chloride had about the same effect as the use of tin-coated cans. The lower level of the stannous chloride additive would be equivalent to about 180 ppm of tin in the product. Concentrations of tin in sweet potatoes resulting from corrosion of tin-coated cans may equal or exceed this level. It has been suggested that tin may replace iron in the complex formed by the quinone reaction resulting in a light yellow colored instead of a dark-colored compound (Hernandez and Vosti, 1963).

The objective values obtained with the Hunter instrument closely followed the visual judgments of appearance, and offered a precise measurement of the change in appearance occurring after exposure of the canned product. Since visual observations of a number of samples could not be made immediately after opening the cans, the visual scores reflect not only the appearance of the product at time of opening but after a

Table 2—Effect of additives on color of sweet potatoes in enamel-lined cans

Treatment <sup>b</sup>	Hunter CDM color difference <sup>a</sup> from control treatment packed in enameled cans						Overall mean
	Syrup			Vacuum			
	Init <sup>c</sup>	1 hr <sup>d</sup>	Mean	Init	1 hr	Mean	
Plain can	4.94 <sup>e</sup>	7.56	6.25	5.63	7.30	6.47	6.36
1½% EDTA	4.27	6.82	5.55	3.48	5.24	4.36	4.96
3% EDTA	1.87	5.49	3.68	2.10	5.66	3.88	3.78
½% SnCl <sub>2</sub>	1.92	4.64	3.28	3.90	6.27	5.09	4.19
1% SnCl <sub>2</sub>	4.24	6.87	5.56	5.83	7.20	6.52	6.04
1% Citric acid	3.53	4.06	3.80	3.65	2.31	2.98	3.39
3% Citric acid	4.82	6.36	5.59	2.12	4.70	3.41	4.50
1½% Ascorbic acid	2.45	3.68	3.07	0.95	1.77	1.36	2.22
3% Ascorbic acid	1.79	3.17	2.48	1.04	(1.24)	(0.10)	1.19
1½% Ascorbic acid + 1½% Citric acid	3.68	2.22	2.95	0.69	(0.58)	0.06	1.50

<sup>a</sup>  $\Delta_E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$

<sup>b</sup> Syrup pack had 10 ml of solution added

<sup>c</sup> Reading taken at time of opening can

<sup>d</sup> Reading taken after 1 hr exposure to air

<sup>e</sup> All values except those in parenthesis indicate higher CDM values than those of control in enamel-lined cans

Table 3—Change in Hunter values upon exposure of canned sweet potatoes as affected by additive treatments

Treatment <sup>a</sup>	Change in Hunter value <sup>b</sup>	
	Syrup pack	Vacuum pack
1½% EDTA	0.37	1.00
3% EDTA	(0.11)	(0.73)
½% SnCl <sub>2</sub>	1.19	0.12
1% SnCl <sub>2</sub>	0.46	1.12
1½% Citric acid	2.53	2.41
3% Citric acid	1.88	1.63
1½% Ascorbic acid	2.08	2.27
3% Ascorbic acid	2.12	3.77
1½% Citric acid + 1½% Ascorbic acid	4.42	3.18
Plain can (control)	0.97	0.98
Enameled can (control)	3.30	3.50

<sup>a</sup> All additive treatments packed in enamel-lined cans

<sup>b</sup> Calculated as  $\Delta_E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$  during 1 hr exposure to air after opening can. Higher values indicate greater darkening during exposure. All values except those in parenthesis indicate darkening during exposure.

Table 4—Effect of additives on visual color score and Hunter CDM values of sweet potatoes processed in enamel-lined cans

Treatment <sup>a</sup>	Visual <sup>b</sup> score	Hunter CDM difference <sup>c</sup>	
		From enamel-lined control	Change upon 1 hr <sup>d</sup> exposure
EDTA 2 ml 3% soln	9.0	5.4	0.7
5 ml 3% soln	9.1	5.5	0.7
10 ml 3% soln	9.0	6.2	1.3
20 ml 3% soln	9.0	6.1	1.3
Mean	9.0	5.8	1.0
Citric acid 2 ml 3% soln	7.9	1.7	2.0
5 ml 3% soln	8.4	1.6	1.9
10 ml 3% soln	8.6	4.1	2.0
20 ml 3% soln	8.9	4.5	0.8
Mean	8.5	3.0	2.3
Sodium bisulphite 2 ml 1% soln	8.4	(1.2)	3.3
5 ml 1% soln	8.3	4.2	3.0
10 ml 1% soln	8.7	5.5	3.9
20 ml 1% soln	8.7	6.3	3.4
Mean	8.5	3.7	3.4
Control (Plain can)	8.5	5.9	1.8
Control (Enamel can)	8.2		2.6
L.S.D. (5%)	0.4		

<sup>a</sup> Amounts given added to No. 303 can

<sup>b</sup> Higher scores indicate less darkening or more attractive color

<sup>c</sup>  $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$ . All values except those in parenthesis indicate higher CDM values than those in enamel-lined cans.

<sup>d</sup> Exposed to air after opening can

could be objectionable and the higher concentrations of citric acid resulted in a detectable acid taste. Although the additive treatments generally resulted in improved product appearance when compared with that processed in enamel-lined cans; the use of plain or tin-coated cans was in most instances nearly as effective as the additives in improving appearance of the product.

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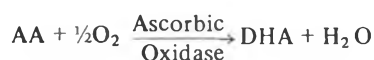
variable exposure time. The retention of color upon exposure resulting from additive treatments as contrasted with the darkening of the product from the enamel-lined control was very apparent in some instances.

None of the additives used in the tests have been approved for use in processed sweet potatoes. Other factors in addition to color would have to be considered. For example, EDTA caused a softening of the exterior of the processed product which

## APPLICATION AND ADVANTAGES OF THE ENZYMIC METHOD FOR THE ASSAY OF ASCORBIC AND DEHYDROASCORBIC ACIDS AND REDUCTONES. Determination in Fresh and Canned Spinach

### INTRODUCTION

METHODS for assaying ascorbic acid based on the reducing properties of vitamin C with regard to 2,6-dichloroindophenol are practical but at the same time limited and not always satisfactory for accurate determination of ascorbic acid in vegetables (Freed, 1966). In particular, after heat treatment it is not always possible to assay accurately vitamin C in preserved vegetables, by titration with 2,6-dichloroindophenol. The cause of possible interferences during the analysis is associated with the presence of reducing substances which are greatly increased during this treatment and of poorly known chemical composition (Numa, 1972). For this reason, the proposed modification of the oxidation-reduction determination is not always effective and applicable to routine analyses (Freed, 1966). We have recently developed an enzymatic method for the assay of ascorbic acid which utilizes purified ascorbic oxidase (Marchesini et al., 1970; Marchesini and Manitto, 1972). This enzyme is, in fact, specific for substrates possessing a cyclic structure characterized by an endiolic system adjacent to a carbonyl group (Dawson, 1966). The method is based on the following reaction:



The ascorbic acid (AA) and dehydroascorbic acid (DHA) content of the vegetable extract is calculated from the oxygen used up during the enzymatic reaction.

The present study, which was carried out on fresh and canned spinach, concerns the evaluation of the limits of the oxidation-reduction method compared with the enzymatic method, paying particular attention to substances interfering in the determination of vitamin C, and of the vitamin C and DHA content in eight different cultivars.

### MATERIALS & METHODS

#### SPINACH (*Spinacea oleracea*)

The tests were carried out on eight varieties cultivated during the autumn. The vitamin C and dehydroascorbic acid were assayed immediately after gathering the spinach. A portion of each cultivar was processed after storage for several hours at 4°C. The operative stages were as follows: washing of the leaves in running water, scalding for 4 min at 92–94°C, washing in running water and canning of the vegetable (280g of blanched tissue per tin). Boiling 2% NaCl solution in water was then added to fill the cans which were heated for 7 min at 75°C in a tunnel heated with free-flowing steam. The cans were then sealed, autoclaved for 27 min at 122°C, and cooled in counterpressure with water against water. The total heat treatment to which the canned spinach was subjected was equivalent to 121°C for 14.7 min. The cans were then stored at room temperature for 5 months.

#### Enzyme: ascorbic oxidase

(AAO) (E.C.1.10.3.3) purified according to Marchesini (1972), has a specific activity of 4000 spectrophotometric units. According to Cohen's (1955) definition, 1 unit is the quantity of enzyme which, placed in a cell (10 mm optical pathway) of 3 ml volume at 25°C, produces a fall in the optical density of 1 unit per minute at 2650 Å. The system originally contains an ascorbic acid concentration equivalent to 1.5 optical density units in 0.1M citrate-phosphate buffer at pH 5.6.

#### Reductic acid

Reductic acid was synthesized from polygalacturonic acid according to the method of Feather and Harris (1966). The compound has a melting point of 212°C (Reichstein and Oppenauer, 1933; 1934).

The following reagents were used as purchased: ascorbic acid, dehydroascorbic acid, potassium metabisulphite, homocysteine, 2,6-dichloroindophenol and solutions containing 1000 ppm of FeCl<sub>3</sub> and CuCl<sub>2</sub>.

#### Preparation of the samples

Two samples (50g each) of fresh or canned spinach were used in parallel, one for the determination of the dry substance and the other, homogenized (Braun knife homogenizer) with 50g of 3% HPO<sub>3</sub> solution containing disodium EDTA, for the present study. 20g of homogenized material were centrifuged at 10,000 × G and 4°C for 15 min. The clear supernatant liquid was collected in glass flasks, covered with aluminum foil and stored in iced-water for the entire duration of the analysis. The oxidation-reduction assay of ascorbic acid was carried out with the 2,6-dichloroindophenol method (Freed, 1966), using a Bausch & Lomb photometer equipped with a digital reader. The enzymatic determination was performed using a Clark's electrode applied to a Gilson recorder.

#### Description of the enzymatic method

The enzymatic method is performed after calibration of the electrode. The two indices—total oxygen and zero oxygen referred to the volume of the cell (Fig. 1)—are obtained with 0.05M phosphate buffer (pH 6.0) saturated with air at 25 ± 0.01°C to which solid sodium dithionite was added. Within the two indices the oxygen uptake is stoichiometrically proportional to the ascorbic acid present in the cell. A control should be run daily with known quantities of ascorbic acid.

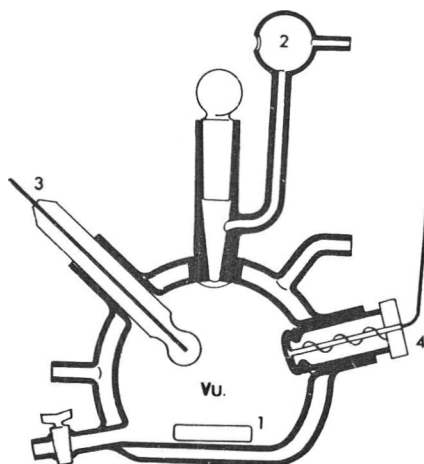


Fig. 1—Cell used in enzymatic method: (1) = magnetic stirrer; (2) = valve for adjusting the N<sub>2</sub> flow-rate; (3) = pH electrode; and (4) = Clark's electrode.

**Table 1—Comparison of the ascorbic acid assay method by the oxidation-reduction technique with the enzymatic method<sup>a</sup>**

	Oxido-reduction mg/100g dry wt	Enzymatic mg/100g dry wt	Δ mg/100g dry wt
Fresh spinach	546	533	13
Canned spinach	245	166	79
Canned spinach <sup>b</sup> + enzyme	56	0	56
Canned spinach <sup>b</sup> + enzyme + 176 mg of ascorbic acid	217	176	41

<sup>a</sup> Results are the mean of 4 determinations. The maximum displacement from the mean is ± 5% for the chemical method and ± 0.8 mg for the enzymatic method.

<sup>b</sup> The extract was brought to pH 6 and the enzyme was added. After 5 min the enzyme was inactivated with metaphosphoric acid and the pH lowered to 2. The extract was then analyzed according to the method described, with or without the addition of ascorbic acid.

**Assay of ascorbic acid**

The assay of ascorbic acid under the analytical conditions used (cell, 17.6 ml volume; temp 25 ± 0.01°C; 10 μl of enzyme equivalent to 1 mg/ml; reaction time 24–36 sec) is carried out between minimum (8 × 10<sup>-5</sup> M) and maximum (5 × 10<sup>-4</sup> M) ascorbic acid values. Within these limits the reproducibility of the assay is ± 8 μg.

Ascorbic acid in vegetables is assayed as follows: the pH of the spinach extract is brought to 6 with 0.21M Na<sub>3</sub>PO<sub>4</sub> phosphate buffer. The remaining space in the cell is filled with 0.05M phosphate buffer (pH 6) and the determination is carried out introducing the enzyme through the top opening of the cell. A given quantity of ascorbic acid is added to the extract to determine the oxygen consumed. This is performed to control for possible inhibitors. A current of nitrogen can exclude all outside interference during the operations.

**Assay of dehydroascorbic acid**

The pH is brought to 6.0 and ascorbic acid oxidase is added; the system is then acidified to pH 2.0.

The pH of the spinach extract is brought to 7.5 with 0.21M Na<sub>3</sub>PO<sub>4</sub> solution and 4 mg of homocysteine is then added. The latter acts as a reducing agent according to the following reac-

tion: Dehydroascorbic acid + Homocysteine (SH) → ascorbic acid. After 15 min the pH is brought to 6 with 3% HPO<sub>3</sub> and the determination is carried out. The value of the ascorbic acid ascertained previously is subtracted from the value obtained (total ascorbic acid).

**Assay of ascorbic acid and dehydroascorbic acid in fresh and canned spinach**

The data are expressed on the dry weight after determining the moisture content of the samples examined.

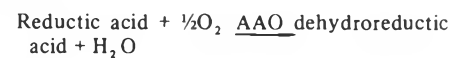
**Specificity of the 2,6-dichloroindophenol and enzymatic methods**

Tests were carried out to assess possible interferences in the assay of ascorbic acid using either the 2,6-dichloroindophenol or the enzymatic method. In both cases the ascorbic acid present was oxidized with ascorbic oxidase. After inactivation of the enzymes, the determinations were carried out on two extracts, one in which no ascorbic acid had been added and one where a known quantity of ascorbic acid had been added. In order to establish the interfering substances found by comparison of the two methods, SO<sub>2</sub>, iron and copper ions, and reductic acid were used.

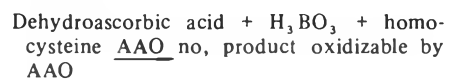
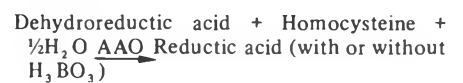
The minimum percentage of substance causing appreciable interference in the assay of ascorbic acid was determined by adding known serial quantities of the single interfering substances.

The reductic acid added to the spinach extract was assayed with the method of Hughes (1956) modified as follows: having assayed the ascorbic acid in the extract the enzyme was inactivated as described above. 4 ml of H<sub>3</sub>BO<sub>3</sub> (1% w/v) were added and the determination was carried out as in the case of dehydroascorbic acid. The value of the oxygen uptake after addition of the enzyme was subtracted from the value of the oxygen obtained in the determination of the total ascorbic acid. Finally, the quantity of reductic acid was calculated stoichiometrically.

The following reactions were therefore utilized:



[The speed of the reaction is slower than that of ascorbic acid (Marchesini and Manitto, 1972).]



Finally, to suppress or check if interferences occurred in the enzymatic determination of ascorbic acid due to the substances added or the heat treatment to which the spinach was subjected, the following reagents were used: 0.5% acetaldehyde for SO<sub>2</sub>, calcium phytate 4 mg per mg of iron, disodium EDTA 10 mg per mg of copper, and 4% w/v H<sub>3</sub>BO<sub>3</sub> 4 ml for reductic acid.

**RESULTS**

TABLE 1 presents results of the vitamin C assay in fresh and canned vegetables using the 2,6-dichloroindophenol oxidation-reduction chemical method and the

**Table 2—Comparison of the interferences in the assay of ascorbic acid using the oxidation-reduction and enzymatic methods. The figures refer to a standard ascorbic acid value (35.2 mg/100 ml) and to 325 mg/100g of fresh spinach tissue (dry weight)**

Assay	Substances added	2,6-dichloroindophenol method		Enzymatic method		Suppression of interferences in the enzymatic assay of ascorbic acid		
		Interfering qty mg/100 ml	Values obtained mg	Interfering qty mg/100 ml	Values obtained mg	mg per 100 ml	mg Ascorbic acid	mg Reductic acid
Ascorbic acid std	SO <sub>2</sub>	0.02	Assayable <sup>a</sup>	8.50	37.8	Acetaldehyde 165 mg	35.2	
	FeCl <sub>3</sub>	0.25	27.8	5.70	37.0	Calcium phytate 20 mg	35.2	
	CuCl <sub>2</sub>	0.25	27.8	1.13	32.6	Na <sub>2</sub> EDTA 20 mg	35.2	
	Reductic acid	0.02	Assayable <sup>a</sup>	0.07	36.8 <sup>b</sup>	H <sub>3</sub> BO <sub>3</sub> 200 mg	—	0.06
Extract of spinach tissue	SO <sub>2</sub>	0.02	Assayable <sup>a</sup>	17	350	Acetaldehyde 165mg	325	
	FeCl <sub>3</sub>	0.50	250	10.40	336	Calcium phytate	325	
	CuCl <sub>2</sub>	0.50	250	2.74	300	Na <sub>2</sub> EDTA 30 mg	325	
	Reductic acid	0.02	Assayable <sup>a</sup>	0.07	330 <sup>b</sup>	H <sub>3</sub> BO <sub>3</sub> 200 mg	—	0.06

<sup>a</sup> Values show wide oscillation.

<sup>b</sup> Two distinct oxygen take-up phases are seen in this determination.

Table 3—Vitamin C and dehydroascorbic acid content of 100g of spinach tissue. Values are expressed on the dry substance

Variety of spinach		Fresh mg/100g of tissue	Canned mg/100g of tissue	Canning liquid mg/100g of tissue
America	AA	528.0	183.2	21.2
	DHA	81.3	25.0	9.1
	AT	549.3	208.2	30.3
	%	100	37.90	5.51
Liscio nero	AA	556.8	202.0	44.5
	DHA	21.3	43.2	8.8
	AT	578.1	255.2	53.3
	%	100	42.42	9.22
Marathon hybrid	AA	549.1	147.0	29.7
	DHA	34.1	25.8	12.2
	AT	583.2	172.8	41.9
	%	100	25.62	7.18
Packer	AA	570.2	149.0	23.2
	DHA	59.5	26.2	7.5
	AT	629.7	175.2	30.7
	%	100	27.82	4.87
Riccio nero	AA	718.0	178.0	32.5
	DHA	51.0	73.2	5.4
	AT	769.0	251.2	37.9
	%	100	32.66	4.92
XP 1029 Hybrid	AA	918.7	179.4	3.7
	DHA	29.0	109.6	17.8
	AT	948.7	289.0	21.5
	%	100	30.46	2.26
Seven R	AA	665.2	119.0	21.9
	DHA	29.0	59.4	11.2
	AT	694.2	178.4	33.1
	%	100	25.70	4.76
Grand Stand	AA	571.1	149.6	32.2
	DHA	25.0	58.0	4.7
	AT	596.1	107.6	36.9
	%	100	34.82	6.19

enzymatic method. Comparison of the ascorbic acid values for fresh spinach, obtained using both methods, gives similar results, with slightly higher values being observed for the chemical method. By contrast, in the case of canned spinach the ascorbic acid values obtained with the chemical method are much higher than those determined enzymatically. Likewise, the results obtained for spinach canned after treatment with ascorbic acid oxidase and addition, or otherwise, of ascorbic acid, give higher values for the chemical method than for the enzymatic method, while the ascorbic acid assayed by the latter method corresponds to the quantity added. No substance assayable with ascorbic acid oxidase was found after oxidation of ascorbic acid by this enzyme.

Table 2 gives results of the ascorbic acid values of a standard ascorbic acid solution and a fresh extract of spinach in the presence of interfering substances. As

can be seen, the chemical assay method is more sensitive to interfering substances. The quantity of sulphur dioxide interfering with the chemical method is 0.2  $\mu\text{g}/\text{ml}$ , compared with 85  $\mu\text{g}$  for the enzymatic method. By contrast, reductic acid interferes with both methods, the interfering level being almost the same in the two cases.

The ferrous ion and Cu show much greater interference in the chemical method.

Table 2 also shows the interferences due to  $\text{SO}_2$  and to heavy metals which may be eliminated with the enzymatic method by adding compounds capable of forming complexes with these substances. On adding boric acid, the interfering reductic acid can be determined directly.

Table 3 gives the ascorbic acid and dehydroascorbic acid content determined with the enzymatic method on eight cultivars. The ascorbic acid values range from 528–919 mg/100g of dry sub-

stance. Dehydroascorbic acid is always present at values ranging from 21–81 mg/100g of dry substance. The ascorbic acid values in canned spinach range from 119–202 mg/100g of dry substance while those for dehydroascorbic acid vary from 25–110 mg/100g of dry substance.

The canning liquid has an ascorbic acid content which fluctuates between 4 and 44.5 mg/100g of dry substance, while the dehydroascorbic acid content ranges from 5–12 mg/100g of dry substance.

## DISCUSSION

ENZYMATIC ASSAY of ascorbic acid in fresh spinach gives values similar to those obtained with the chemical method, although slightly inferior.

There is a distinct difference in the case of canned spinach. The values for ascorbic acid are always significantly lower than those obtained with the chemical method. It is known that 2,6-dichloroindophenol oxidizes ascorbic acid more rapidly compared with the other reducing substances present in vegetable extracts. In fact, photometric chemical analysis necessitates limiting the reaction time and a correction calculated in a given time so as to eliminate the interferences due to reducing substances other than ascorbic acid. However, these corrections are not always satisfactory for a specific assay of ascorbic acid in vegetable extracts. This fact is particularly apparent in the case of heat treated vegetable extracts. In the case of the enzymatic method it is possible to demonstrate that no natural substances of inhibiting action are present in spinach extract.

Assay of the ascorbic acid in the extracts, performed after oxidation of the ascorbic acid naturally present, and with addition of pure ascorbic acid, has given the quantity of ascorbic acid added, thus excluding the action of inhibiting substances. The eight varieties of spinach—both fresh and canned—cultivated in the autumn, show good vitamin C and dehydroascorbic acid contents. "Liscio nero" is the richest variety with 910 mg/100g of tissue.

Heat treatment leads to a loss in the amount of vitamin; after 5 months the ascorbic acid values are around 120–202 mg/100g of tissue.

The presence of reductic acid in spinach subjected to heat treatment was never observed in any of the tests. In conclusion, the enzymatic method is specific and can be used gainfully for routine analysis of ascorbic and dehydroascorbic acids.

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## EXTRUSION PROCESSING OF TRITICALE

### INTRODUCTION

TRITICALE is a man-made cereal grain. The parental species are wheat and rye. The objectives of this cross were to combine grain quality, productivity and disease resistance in triticum (wheat) with the vigor and hardness in secale (rye).

The major importance of triticale lies in its protein. Protein content and quality varies depending upon environmental conditions and fertilization. Triticales have exhibited a wide range of protein content from 12–22% (Pomeranz, 1971; Zillinsky and Borlaug, 1971a).

Rye protein seems to be more nearly balanced in amino acids than wheat protein. This trait with a slightly higher lysine content is inherited in triticale (Pomeranz, 1971).

Triticale protein seems to be more digestible as evidenced by feeding experiments involving sheep, cattle and rats (Zillinsky and Borlaug, 1971b; Busch and Wilkins, 1972). A study with adult humans compared protein retentions on diets of triticale and wheat grains. At two levels of protein intake, triticale showed a higher protein retention (Kies and Fox, 1970b).

Highly acceptable breads can be produced from triticale flours (Lorenz, 1972; Lorenz et al., 1972a; Tsen et al., 1973). The production of hard rolls and white rye breads has also been shown to be possible (Lorenz, 1972). To demonstrate further use for triticale flour, pasta products such as noodles were processed from triticale and compared with those made from semolina. Triticale noodles required less time to cook and had a greater cooking tolerance than other flour noodles (Lorenz et al., 1972b).

Triticale may find use as a malt supplement to low-sugar dough (Finney et al., 1972). The high enzymatic activity of triticale may prove to be of use in the brewing industry (Pomeranz, 1971).

In applications relating to cereal products, work on extrusion of triticale is presented here, because extrusion cooking of cereals is an increasingly important food operation (Harper, 1971). High temperature-short time (HTST) processing is a characteristic of extrusion. Math-

ematical models of the extrusion process (Harmann and Harper, 1972; Harper et al., 1971; Schenkel, 1966; Tadmor and Klein, 1970) describe the feed, transition and metering zones of the extruder. Pressures and temperatures are highest in the metering zone, hence the greatest changes in triticale properties may occur there. Empirical models are developed here relating texture score, flavor score, moisture content after extrusion, color, torque and pH to the independent variables: moisture content before extrusion, temperature and nozzle size.

### MATERIALS & METHODS

#### Sample description and preparation

The triticale was obtained from a commercial source and was grown in Colorado. Moisture and ash of the cereal grain were determined by AOAC (1960) procedures and protein content by the Udy Protein Analyser method (AACC, 1962). The protein content of the grain was 14.5% and the ash content 1.9% on a 14% moisture basis.

Experience with other grains indicated that 20% moisture might make for easy extrusion. Samples of the whole triticale grain were tempered for 1 day to 15%, 20% and 25% moisture in sealed containers. The whole grain was then fed to the extruder.

Another sample was mixed with water to bring the moisture content to 25%, and immediately tumbled and blended in a Patterson-Kelley liquid-solids twin shell blender model LB-P-8 for 20 min. This loosened and removed the bran portion from the triticale kernels. After storing in sealed containers for 24 hr, the bran portion was separated by forced air. Moisture content of the dehulled material was 22%.

#### Extruder description

The whole and debranned triticale kernels were extruded with a Brabender Plasticorder extruder. A 3/4 in. rifled barrel was used. Nozzle openings of 1/8 in. and 1/16 in. were used. The extruder barrel is divided into two temperature controlled zones. Both are equipped with electric heaters and air cooled jackets for temperature control. A third temperature controlled zone is the transition section from the extruder barrel to the nozzle. It is equipped with an electric heater and is cooled by ambient convection.

#### Preliminary experiments

Preliminary experiments were made using 1:1, 3:1 and 5:1 screws and 1/8 in. and 1/16 in. nozzles. It was found that the triticale did not feed well through the extruder using the 3:1 and 5:1 screws. The triticale was ground by the screw and was pushed back into the feed

hopper because of the considerable amount of steam generated. The steam caused caking and made smooth operation impossible.

Triticale samples of as low as 7% moisture content initially were extruded. Even at this low moisture content, the torque remained low. However there was almost no puffing regardless of the temperature used. The product was hard, and as the temperature was increased from 300°F to 450°F the product became scorched. At the lower extrusion temperatures, the product became soggy as the moisture content was increased. With increasing temperature, the increased moisture content of the feed material produced a puffed product. The higher moisture content also retarded scorching.

The product did not flow from the extruder in a continuous piece, but was emitted from the nozzle as bursts of individual particles. Since whole grains were fed exclusively in these preliminary experiments, the presence of the hulls created a nonhomogeneous material coming from the extruder. It appeared that a hull would stick in the nozzle until the pressure built up sufficiently to dislodge it. Then material near the end of the extruder would expand rapidly, producing a rapid outflowing of material which fragmented into individual particles as it emerged from the nozzle. When the pressure had lowered sufficiently, another triticale hull would lodge in the nozzle and stop the flow. Then the cycle would repeat itself. This produced an effect reminiscent of a machine gun. Later, when debranned material was used, operation was much more uniform.

In general it was found that the higher the extrusion temperature the higher the moisture content needed to avoid a scorched product. It was noted that these higher temperatures and moisture contents produced a more well puffed product. It was also noted that at the higher temperatures more water was required to go beyond the well puffed product, producing a soggy product.

The preliminary experiments indicated that a moisture content of 20% and a temperature of 400°F were approximately the best conditions. Further, some second order effect was noted, since either high or low temperature or high or low moisture could produce undesirable effects. Accordingly an experimental design was chosen with three levels of temperature, 350°F, 400°F and 450°F in zones 2 and 3, and three levels of moisture, 15%, 20% and 25% to allow estimation of second order effects in the empirical statistical model for these independent variables. It was expected that nozzle diameter would have a linear effect, so only two levels, 1/8 in. and 1/16 in., were used in the design. In actual experimentation it was not possible to obtain sufficient good samples at 25% moisture due to caking problems in the feed. Therefore this level was dropped. The final design for whole grain samples is shown in Figure 1, which is similar to designs discussed by Box and Draper (1969).

<sup>1</sup> Dept. of Food Science & Nutrition

<sup>2</sup> Dept. of Agronomy

<sup>3</sup> Dept. of Agricultural Engineering

The extruded samples were analyzed for moisture content as outlined earlier. Torque measurements represent average values while the samples were being processed.

**Color measurement**

Samples of extruded triticale were ground with a Wiley #4 Laboratory Mill using a screen with 1 mm openings. Ground samples were placed in 50 ml beakers and the color of the samples determined with a Hunter Color Difference Meter Model D25.

**Panel evaluation**

The extruded products were evaluated for texture and flavor by an untrained test panel consisting of ten college students and faculty members. The panel was instructed to evaluate the products using the following rating scale: 1-like very much, 2-like slightly, 3-indifferent, 4-dislike slightly and 5-dislike very much.

**Trypsin hydrolysis**

Kies and Fox (1970 a, b) have shown that humans on diets of triticale have a higher protein retention than on diets of other cereal grains. There is, however, the possibility that extrusion of triticale will affect the protein of the grain, therefore influencing the digestive acceptability. It has been suggested by Tremolieres (1970) that digestive acceptability of a protein can be determined by measuring the intensity and rate of proteolysis with trypsin.

The in vitro method of enzyme hydrolysis used was a modification of that reported by Maga et al. (1973). Extruded, ground samples of 1g each on a moisture-free basis were suspended in 20 ml of distilled, demineralized water and the pH of the suspensions adjusted to 7.0 with 0.1N NaOH. Samples were permitted to rehydrate with intermittent shaking for 1 hr at approximately 4°C. The samples were then incubated at 37°C for 20 min, after which 2 ml of lyophilized trypsin at a concentration of 1500 units per ml was added. Incubation mixtures were withdrawn after 1, 2, 3, 5 and 7 min and the pH immediately recorded. Results are based on duplicate determinations.

**RESULTS**

THE DATA and physical description of the samples are listed in Table 1. The data in Table 1 were divided into two groups: whole grain and debranned grain extruded samples. Since only one moisture level was investigated for debranned grain, its design has only two independent variables. Both designs were analyzed using:

$$\beta_i = [X^T X]^{-1} [X^T Y_i]$$

Table 2 shows the estimated values of  $\beta_i$  from the above equation for the whole grain data and for debranned grain data. The empirical mathematical models are respectively, whole grain:

$$\begin{aligned} \hat{Y} = & \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 \\ & + \beta_{22} (X_2)^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 \\ & + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \\ & + \beta_{122} X_1 (X_2)^2 + \beta_{322} X_3 (X_2)^2 \\ & + \beta_{1223} X_1 X_3 (X_2)^2 \end{aligned}$$

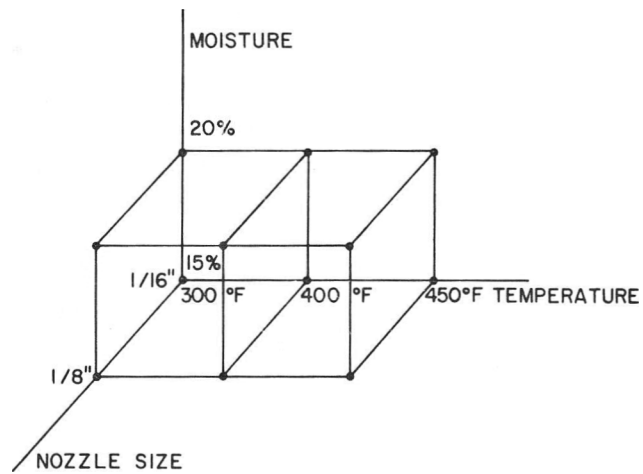


Fig. 1—Mixed two and three level full factorial experimental design of triticale extrusion experiments.

where  $X_1 = (\%H_2O - 17.5)/2.5$ ;  $X_2 = [T(^{\circ}F) - 400]/50$ ; and  $X_3 = [Nozzle diameter (in.) - 0.09375]/0.03125$ ; and debranned grain:

$$\hat{Y} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} (X_1)^2 + \beta_{12} X_1 X_2 + \beta_{112} X_2 (X_1)^2$$

where:  $X_1 = [T(^{\circ}F) - 400]/50$  and  $X_2 = [Nozzle diameter (in.) - 0.09375]/0.03125$ .

The coefficients of the empirical model are useful to help pinpoint the important variables. No attempt will be made here to optimize the process based on different priorities or weights of the dependent variables.

**Texture and flavor scores**

Texture score coefficients for whole

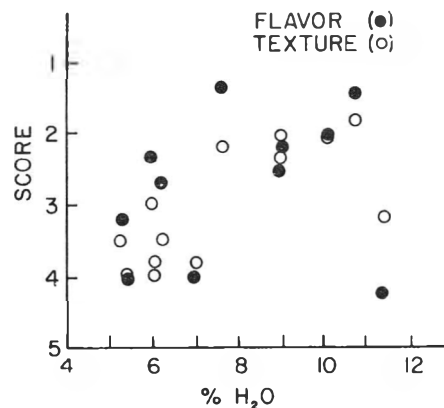


Fig. 2—Flavor and texture score of whole grain triticale correlated with percent water content after extrusion.

grain and debranned grain gave conflicting results for the linear terms on temperature and nozzle size, but both show positive second order effect for temperature. This indicates an optimum mid-range. Further insight on the quadratic nature of texture and flavor scores can be seen from Figure 2 which shows improvement then decreases. Moisture content after extrusion is primarily linear with respect to the independent variables.

The nozzle diameter affected the size of the particles of the extruded breakfast cereal. Satisfactory products were extruded with each nozzle as seen in Table 1. Personal preference will dictate the diameter of the extruder nozzle to use.

The debranned grains generally produced larger particles than the whole triticale grains. The debranned extruded grains had a dull brown color and were less appealing than the extruded whole grain kernels.

Using the small diameter nozzle (1/16 in.), golden brown, crunchy and crisp particles were extruded at 350°F with the grains tempered to 15% and 20% moisture. The sample of triticale which had been tempered to 20% received the best flavor and texture score (2.0) indicating an acceptable product. Extruding the same sample of triticale at 400°F and 450°F resulted in slight scorching which greatly affected the flavor score of the products. Using the larger nozzle (1/8 in.), the best products were extruded at a temperature of 400°F with the samples tempered to 15% and 20% moisture. The 20% moisture sample produced the best overall product—a golden brown, crunchy and crisp product. Increasing the temperature to 450°F resulted in a light, fluffy, but scorched product.

Table 1—Experimental data

Sample <sup>a</sup>	Percent water before extrusion	Temp. in heating Zones 1 & 2 (°F)	Nozzle diam. (in.)	Texture score $Y_1$	Flavor score $Y_2$	Percent water after extrusion $Y_3$	Color <sup>b</sup>			$\Delta E$ $Y_4$	Torque Meter (g X 0.1) $Y_5$	pH $Y_6$	Product characteristics
							L	a	b				
Whole grain	15	350	1/16	2.5	2.3	9.0	61.3	3.3	11.8	33.3	360	6.7	Golden brown color
Whole grain	20	350	1/16	2.0	2.0	10.1	58.7	3.2	12.8	36.1	200	6.55	crunchy, small particles Golden brown color
Debranned	22	350	1/16	4.0	2.8	11.0	63.7	3.1	11.0	30.8	160	6.7	crunchy Dull brown color
Whole grain	15	400	1/16	2.3	3.0	6.0	58.1	5.1	15.3	37.7	100	5.6	large particles Light brown color
Whole grain	20	400	1/16	4.0	3.8	7.0	57.0	6.0	15.2	38.8	90	5.75	very small, crisp particles Light brown color
Debranned	22	400	1/16	3.0	2.3	10.2	63.5	1.8	9.2	30.3	120	6.3	slight scorched, small particles Dull brown color
Whole grain	15	450	1/16	3.2	3.5	5.3	55.6	5.3	15.6	40.0	100	5.25	Large particles, tough Fluffy product, light brown color, slightly scorched
Whole grain	20	450	1/16	2.7	3.5	6.2	56.2	5.6	15.9	39.7	80	5.5	Very light product, fluffy scorched
Debranned	22	450	1/16	2.7	3.8	8.0	64.4	4.6	12.8	30.9	120	6.15	Dull brown color, fluffy scorched
Whole grain	15	350	1/8	2.2	2.0	9.1	61.7	4.6	10.2	32.6	120	6.6	Dark brown color, dull, heavy, large particles
Whole grain	20	350	1/8	4.2	3.2	11.4	59.9	3.6	10.5	34.6	100	6.5	Large particles, heavy, dense
Debranned	22	350	1/8	4.2	3.2	13.6	64.0	3.0	11.0	30.5	140	6.6	Large particles, dull brown color, tough
Whole grain	15	400	1/8	1.4	2.2	7.6	58.2	4.0	13.9	37.0	80	6.4	Golden brown color, crunchy
Whole grain	20	400	1/8	1.4	1.8	10.8	60.7	4.2	11.2	33.8	80	6.15	Golden brown color, crunchy
Debranned	22	400	1/8	3.8	2.7	13.1	60.2	2.9	13.0	34.7	120	6.5	Dull brown color, heavy, dense, tough
Whole grain	15	450	1/8	4.0	4.0	5.4	57.1	4.4	14.2	38.7	60	5.45	Dark brown color, scorched, light
Whole grain	20	450	1/8	4.0	3.8	6.1	54.2	5.3	15.6	41.4	50	5.65	Dark brown color, scorched, light
Debranned	22	450	1/8	3.5	2.5	11.4	63.0	4.2	13.2	32.3	110	6.5	Dull brown color, slightly tough, slightly heavy

<sup>a</sup> Samples extruded at 75 RPM

<sup>b</sup> Standard values:  $L = 92.7$ ;  $a = -0.8$ ;  $b = -1.0$ ;  $\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$

**Moisture content after extrusion**

As would be expected, higher extrusion temperatures resulted in lower moisture content in the extruded product, with the second order coefficient being not significant. A nozzle diameter increase yields higher moisture content as seen from the beta values in the results.

**Color**

Color became darker with increasing temperatures as can be seen from the beta values while increasing moisture lightened the color of whole grain samples. Nozzle size gave conflicting results. Second order effects were of low significance, but indicated a minimum in color at the mid-range of temperature.

**Torque**

Increased temperature significantly lowered torque and seemed to be the most important variable affecting torque. Some second order effect of temperature appears in the results, probably due to the much higher torque values at 350°F.

**pH**

As with torque, pH was most strongly decreased at increased temperature, but second order effect was minimal.

**Trypsin hydrolysis**

The rates of trypsin hydrolysis of extruded samples changed very little as the temperature in zones 2 and 3 of the ex-

truder increased from 350°F to 450°F. There appears to be a greater effect on trypsin hydrolysis rates due to the size of the nozzle in extrusion than due to the temperature of extrusion. Therefore only the hydrolysis rates of products extruded at 400°F with the two different nozzles were considered.

After initial adjustment of the pH to 7.0, there was a slightly faster rate of hydrolysis of the suspensions where the grain had been tempered to 20% moisture, compared with samples tempered to only 15% moisture. This was found to be true with both extrusion nozzles. The larger nozzle (1/8 in.), however, produced products which showed a greater rate of hydrolysis than those made with the smaller nozzle. Samples extruded with the smaller nozzle are subjected to higher pressure during extrusion, thereby affecting the enzyme hydrolysis rates of the protein. Higher moisture contents (20% vs. 15%) seem to protect the protein from changes due to temperature and/or pressure during extrusion.

The samples tempered to 22% have been considered separately, since these samples were debranned before extrusion. The same conclusions, however, can be drawn as for the whole triticale kernels. Products extruded with the larger nozzle showed faster trypsin hydrolysis rates that those produced with the small nozzle.

Trypsin hydrolysis rates of products extruded with the small nozzle (1/16 in.) were slower than those for the unprocessed, ground triticale grain. Using the large nozzle, these rates were comparable.

**CONCLUSIONS**

THE MOST ACCEPTABLE products, considering texture, flavor and color, were extruded using the whole triticale kernels tempered to 20% moisture and extruded at 350°F with the small nozzle or tempered to 20% moisture and extruded to 400°F using the larger nozzle.

It is concluded that triticale tempered to 15% to 20% moisture and extruded at 350°F, 400°F and 450°F does not change in digestive acceptability when the large nozzle is used. Rates of trypsin hydrolysis are slower with the smaller nozzle (1/16 in.).

Heat usually either makes protein more susceptible to hydrolysis due to structural changes or by acting on residual materials in a product such as lipids and carbohydrates which could have been binding a portion of the protein (Maga et al., 1973). The beneficial effect of heating shown with certain high protein additives (Maga et al., 1973) was not observed in the extrusion of triticale. The relatively short residence time of the products in the heating zones of the extruder (approximately 1 min) apparently did not

Table 2—Results for whole grain and debranned triticale

	Texture model	Flavor model	Moisture after extrusion model	Color model	Torque model	pH model
	$\beta_1$	$\beta_2$	$\beta_3$	$\beta_4$	$\beta_5$	$\beta_6$
<b>Whole grain triticale</b>						
$\beta_0$	2.275	2.7	7.85	36.825	87.5	5.975
$\beta_1$	0.425	0.1	1.05	-0.525	-2.5	-0.025
$\beta_2$	0.375	0.6625	-2.075	2.825	-61.25	-0.5625
$\beta_3$	-0.875	-0.7	1.35	-1.425	-7.5	0.3
$\beta_{2,2}$	0.825	0.3375	-0.025	-0.15	46.25	0.05
$\beta_{1,2}$	-0.25	-0.1375	-0.225	-0.225	18.75	0.0875
$\beta_{1,3}$	-0.425	-0.3	0.55	-1.075	0.0	-0.1
$\beta_{2,3}$	0.025	-0.0125	-0.175	0.25	33.75	0.0625
$\beta_{1,2,3}$	-0.25	-0.2125	-0.175	0.55	-16.25	-0.0125
$\beta_{1,2,2}$	-0.3	-0.0125	-0.425	1.5	-23.75	0.05
$\beta_{3,2,2}$	1.375	0.9125	-1.175	1.125	-43.75	-0.275
$\beta_{1,2,2,3}$	0.8	0.4625	-0.425	1.425	18.75	0.1
<b>Debranned triticale</b>						
$\beta_0$	3.4	2.5	11.65	32.5	120.0	6.4
$\beta_1$	-0.5	.075	-1.3	0.475	-17.5	-0.1625
$\beta_2$	0.4	0.2	1.45	2.2	0.0	0.1
$\beta_{1,1}$	0.2	0.575	-0.65	-1.375	12.5	0.0875
$\beta_{1,2}$	0.15	-0.425	0.2	0.425	2.5	0.1125
$\beta_{1,1,2}$	-0.15	-0.425	-0.05	-1.925	-7.5	0.0375

affect significantly the protein of triticale.

A very acceptable breakfast cereal can be produced by extrusion processing of tempered whole grain triticale. With proper conditions the digestive acceptability of the proteins in triticale remained unchanged during the high temperature-short time extrusion process.

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## WHOLE SOYBEANS AS A MEANS OF INCREASING PROTEIN AND CALORIES IN MAIZE-BASED DIETS

### INTRODUCTION

RESULTS OF recent studies carried out in 4-month-old dogs fed maize-bean diets, as consumed by small children in many rural areas of Latin America, showed that protein and calories (as oil) added individually or together, improved the utilization of the protein of the maize-bean diet (Murillo and Bressani, 1972). These findings confirmed the fact that diets of this type are deficient in both calories and good-quality protein. Used by themselves, they cannot provide the necessary calories because of their low calorie density and their great bulk, and also because for economic reasons consumers of these diets are unable to purchase greater amounts of such foods.

Previous studies carried out by Elías and Bressani (1972) have concentrated on providing more and better-quality protein. The present article deals with the results obtained by using a single food to provide more protein of better quality and more calories. Soybean was the food chosen for this purpose after subjecting it to the cooking treatment maize receives before consumption in the area.

### MATERIALS & METHODS

THE MAIZE selected for these studies was the common variety grown at the INCAP experimental farm located at an altitude of 5,350 feet above sea level. The soybean Pelican variety used was also grown on the same farm. This material contained 14.0% fat and 30.3% protein.

Processing of both maize and soybeans was carried out by the lime-cooking process commonly used to prepare tortillas (flat-cooked pancakes) as previously described (Bressani et al., 1958). After cooking and washing, the grains were ground wet in a rotary mill. The resulting dough was spread on a tray and dried using warm air at 65°C. Once dried, the material was then ground to 40 mesh.

Some of the dough was made into tortillas and then dried and ground. All samples were analyzed for their proximate chemical composition according to AOAC standard methods of analysis (AOAC, 1960).

The process was applied to prepare two types of foods, wherein both maize and soybeans were cooked together. The proportions of the two materials differed according to the type of food preparation desired. These are described under Results. Water retention capacity of the supplemented and unsupplemented tortillas was determined by continuous weigh-

ing of both types of preparations over a period of 3 days. Tortillas were then left on a laboratory bench, in a room at a temperature of 22°C.

The final dried flour form preparations were then submitted to biological assays for protein quality, using the PER method. In these assays, both protein level of the diet and fat content were adjusted so as to make valid comparisons. The different preparations were added to a basal diet containing 5% oil, 4% mineral mixture (Hegsted et al., 1941), 1% cod liver oil and 5 ml of a complete vitamin-B solution (Manna and Hauge, 1953). When necessary, diets were adjusted to 100% with maize starch. Each experimental group was made up of 10 weanling rats, placed in individual all-wire cages, with raised screen bottoms. Food and water were provided ad libitum and weight changes were measured four times every 7 days.

### RESULTS

#### Preparation of soybean-enriched tortillas

Previous studies had indicated that the addition of 8% soybean flour, containing 50% protein, gave optimum protein quality when added to maize (Bressani and Marengo, 1963). Since 8% soybean flour provided 4g of protein, quantities supplying this additional amount were used in our studies with whole soybeans. The level chosen was rounded to 15% and added to 85% of raw, yellow or white maize, following the procedure described in Figure 1. To each batch of maize and whole soybeans were added 1.6 times water, by weight, and 1.7% calcium

Table 1—Balance of materials for tortilla preparation

Mixtures used		Amt cooked (kg)	Amt of dough recovered (%)	Amt of tortilla recovered (%)
Maize (%)	Soybean (%)			
100	0	8.0	87.5	—
85	15	8.0	89.3	—
100	0	2.0	—	79.6
85	15	2.0	—	84.5

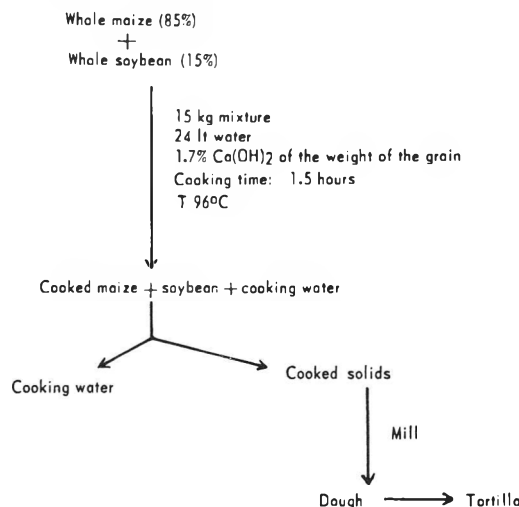


Fig. 1—Tortilla preparation from maize and whole soybeans.

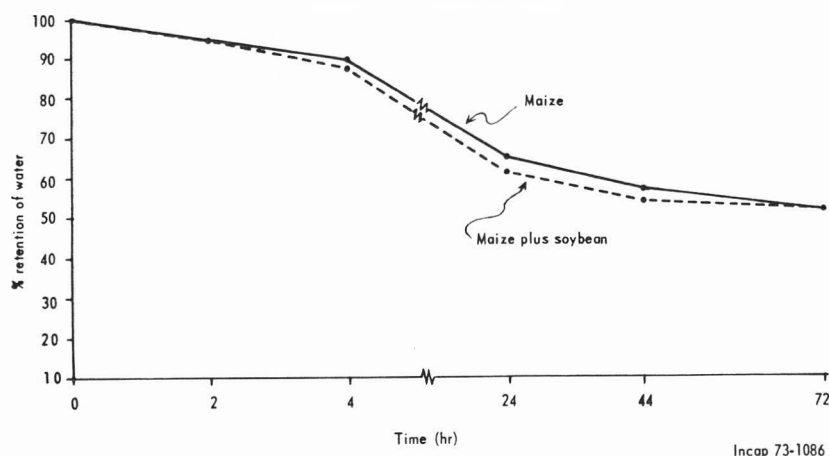


Fig. 2—Water-retention capacity of tortillas made from maize, and from maize plus soybeans (22°C).

hydroxide. Grains were cooked for 1.5 hr, the time needed to reach boiling temperature. They were then cooled to room temperature, the cooking liquor discarded, and the grains washed three times with water. Immediately afterwards the cooked material was ground and weighed, and prepared into tortillas in order to measure their yields. These results are shown in Table 1. Slightly higher yields of both dough and tortillas were obtained when maize was processed with 15% whole soybeans. The loss in yield occurs because small amounts of dough remain attached to the mill and part is also lost during actual preparation of tortillas. Table 2 shows the chemical com-

position of these preparations. Data reveal that when they contained 15% soybeans, their protein content increased from 10 to around 14%. Likewise, crude fiber, ash and fat contents increased with the presence of soybeans. Water retention of common tortillas and of those made from maize plus soybeans was essentially the same over a 12-hr period at room temperature (Fig. 2).

Data concerning protein quality of these preparations are presented in Table 3. Results show that protein quality was higher for preparations containing soybeans as whole beans or as flour, tested at 9 or 12.5% protein in the diet, and that oil in the soybean-corn preparation also

contributed to its nutritive value.

#### Preparation of a high-protein food from corn and whole soybeans

Mixtures of corn and soybean flour of maximum protein quality (Bressani and Elías, 1966) served as the basis for the studies reported herein. Those mixtures where each component supplied 50% of the total protein, gave maximum protein efficiency ratios and were used for the formulation of INCAP Vegetable Mixture 14 (Bressani and Elías, 1966). In the present studies, whole maize and soybeans alone and in various combinations were processed by the lime-cooking treatment described in Figure 3. The resulting dough was dried, weighed, analyzed and used for biological tests.

Table 4 summarizes the yields obtained, and results demonstrate that as the amount of soybeans increased in the preparation to be cooked, yield of these materials decreased significantly. The protein and fat content of the various preparations, as well as the weight gain of the rats consuming them, together with their protein efficiency ratio, are summarized in Table 5. As expected, protein and fat content increased as the amount of soybeans increased. Weight gain also improved, but the increments obtained when the amount of soybeans in the mixture was augmented, decreased after the mixture exceeded 28% soybeans and 72% corn, respectively. This preparation gave the highest protein efficiency ratio.

## DISCUSSION

RESULTS OF the present study indicate that it is possible to prepare tortillas

Table 2—Chemical composition of dough and tortillas made from maize-soybean mixture (expressed in percentage)

	Moisture	Fat	Crude fiber	Protein	Ash	Moisture (fresh)
Dough, white maize (dehydrated)	6.5	3.0	1.6	10.0	1.8	—
Dough, white maize + soybean (dehydrated)	5.5	5.5	2.7	13.9	3.2	—
Tortilla from white maize	1.4	2.2	2.0	10.4	1.6	53.0
Tortilla from white maize + soybean	1.4	4.1	2.8	14.4	2.3	53.8

Table 3—Protein quality of maize dough prepared with or without soybeans

	Avg wt Protein (%)	Avg wt gain (g)	PER	Relative nutritive value	Utilizable protein (%)
White maize dough <sup>a</sup>	9.0	18	0.95	23.7	2.14
85% white maize dough + 15% soybeans <sup>a</sup>	9.0	40	1.72	43.0	3.87
92% white maize dough + 8% soybean flour supplement <sup>a</sup>	9.0	40	1.70	42.5	3.82
Casein <sup>a</sup>	9.0	112	3.00	75.0	6.75
85% white maize dough + 15% soybeans <sup>a</sup>	12.5	84	1.98	57.1	7.14
92% white maize dough + 8% soybean flour supplement <sup>a</sup>	12.0	68	1.98	57.1	6.58
Casein <sup>a</sup>	12.5	124	2.60	75.0	9.38
85% white maize dough + 15% soybeans without 5% added oil in diet	12.5	78	1.98	57.1	7.14

<sup>a</sup> All diets were supplemented with 5% soybean oil.



based on mixtures of 85% maize and 15% whole soybeans with a protein quality and nutritive value superior to that of the tortilla made from maize alone. Obviously, this increase is due to the contribution of the limiting amino acids in the maize protein—lysine and tryptophan (Bressani et al., 1968)—by the addition of whole soybeans, as well as to the higher levels of fat attained also through these means. The tortillas had similar physical characteristics, and water retention for both types of preparations was essentially the same. Furthermore, acceptability was very good among testers. These findings were to be expected, since previous research using soybean flour had shown an increase in protein quality (Eliás and Bressani, 1973) and good acceptability of the product (Eliás and Bressani, 1971). The difference, however, lies in the fact that when tortillas are made with whole soybeans, they contain a relatively higher level of calories, nutrient that is also deficient in the habitual diet of children and adults of poor economic resources in the Central American countries (INCAP, 1969). The biological tests conducted, proved that the addition of calories is an important factor in improving the nutritive quality of the tortilla prepared with soybeans, a finding that corroborates results of previous studies (Murillo and Bressani, 1972). In terms of total intake of protein and calories, consumption of tortillas with whole soybeans would be preferable to an increased intake of simple tortillas, since with the latter it would be difficult to obtain the calorie intake and protein quality actually needed to improve the nutritive quality of the diet.

According to results presented in this study, products with higher protein content and energy value can also be obtained. A mixture of 72% maize and 28% whole soybeans, with 18% protein and 10% fat would be a good-quality food preparation for small children. Protein-rich foods with the same protein levels are now being distributed in developing countries (Bressani and Eliás, 1973) to

help alleviate protein-calorie malnutrition. Further studies with the mixture of 72:28 maize:soybeans, named "Maisoy," are now under way.

An interesting finding is that as the amount of soybeans increased, yields of cooked solids decreased. This was attributed to the solubility of the soybean protein in alkaline solutions. The fact that rats grew relatively well, therefore suggesting that the lime-cooking process inactivated the antiphysiological factors present in soybeans, is another important finding (Liener et al., 1958). Nevertheless, both aspects deserve further study.

Besides, it will be necessary to test the stability of the 72:28 food preparation because of the relatively large amount of fat present in it, and considering the possible effects of the alkaline conditions used. High lipid acid values of lime-cooked maize can be detected after 3 to 4 months of storage (Bressani et al., 1962); therefore, it is possible that this may also

occur with the 72:28 maize:soybean mixture in the dehydrated form and under certain storage conditions.

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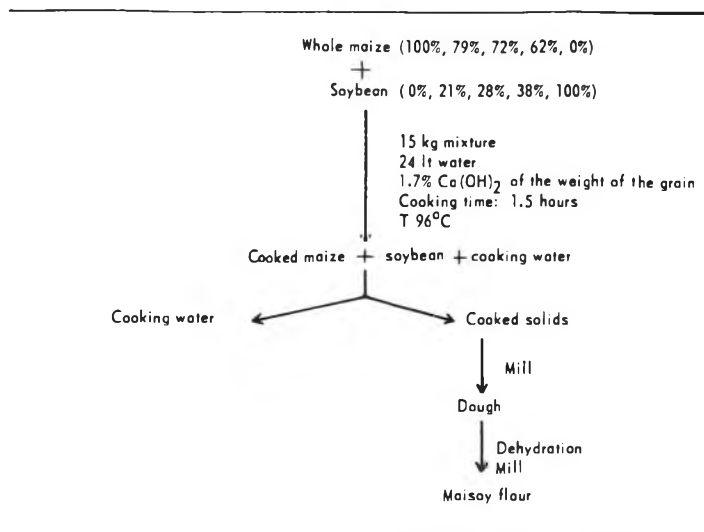


Fig. 3—Processing of mixtures of maize and soybeans.

Table 4—Balance of material of various maize-soybean mixtures

Mixture		Amt processed (kg)	Amt recovered (kg)	Yield (%)
Maize (%)	Soybean (%)			
100	0	15.0	12.0	80.0
79	21	15.0	12.2	81.1
72	28	15.0	12.0	80.0
62	38	15.0	10.8	72.0
0	100	15.0	9.4	62.7

Table 5—Protein and fat content and protein value of the different preparations<sup>a</sup>

Mixture		Content of		Avg wt gain (g) <sup>b</sup>	PER
Maize (%)	Soybean (%)	Protein (%)	Fat (%)		
100	0	9.9	4.5	12	0.69
79	21	16.9	8.9	81	2.08
72	28	17.6	10.3	91	2.54
62	38	18.1	11.3	99	2.37
0	100	40.0	25.6	101	2.03
Casein		—	—	120	2.87

<sup>a</sup> All diets were calculated to contain 9% protein.

<sup>b</sup> Average initial weight: 47g.

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## NUTRITIVE VALUE OF A FIBER-FREE COCONUT PROTEIN EXTRACT OBTAINED BY AN ENZYMIC-CHEMICAL METHOD

### INTRODUCTION

COCONUT PROTEINS have been shown to have a relatively favorable amino acid profile (Tasker et al., 1962; Srinivasan et al., 1964) and a fairly high nutritive value (Krishnamurthy et al., 1958; Loo, 1968). However, the protein quality of coconut meal (defatted coconut flour or copra meal) has been shown to be compromised by the relatively high crude fiber content of the meal itself (Rama Rao et al., 1965) and by the heat treatment which the copra is commonly subjected to either before or after the oil extraction (Better and Davidsohn, 1958; Butterworth and Fox, 1963). Such factors also limit considerably the use of coconut meal in diet formulations, especially when such diets are intended for children (Teply and Gyorgy, 1962; Daniel et al., 1968).

Although several methods have been devised for the protein extraction of coconut meal (Chandrasekaran and King, 1967; Chelliah and Baptist, 1969; Rama Rao, 1969; Samson et al., 1971) almost no data are found in the literature concerning the nutritive quality of the proteinaceous material extracted by the different techniques.

The proteins of the coconut meal obtained through the azeotropic oil extraction process have been reported to be 80% water extractable (Rajasekharan, 1967). Rama Rao et al. (1964) reported a protein efficiency ratio (PER) of 1.88 for these water soluble proteins when the extract was dried at 60°C. A PER of 1.86 was found by these authors for the proteins of the extract that precipitated at pH 4.0.

Chandrasekaran and King (1967) reported a PER of 1.81 for their enzymatically extracted protein and a PER of 2.27 for the protein extracted without enzyme. Statistically the difference was reported to be not significant; however, the authors stated that those results were based only on the performance of the animals between the 8th and 15th days of the test.

The aim of the work described in the present paper was to determine the nu-

tritive value of the fiber-free protein extracted from a commercial coconut meal by a simple, enzymic-chemical method devised in our laboratories (Molina and Lachance, 1973) and to establish to which extent the fiber content compromises the nutritive value of the proteins in the original coconut meal, and when added to the extracted material.

### EXPERIMENTAL

#### Nutritive value

*Chemical evaluation.* Both, amino acid analyses and protein score calculations, were carried out for a commercial food grade coconut meal from Heyman Process Corp. (Long Island City, N.Y.) and for the extract obtained at the pilot plant scale (Molina and Lachance, 1973) from the Heyman meal by the enzymic-chemical method.

The amino acid analyses were performed by the Wisconsin Alumni Research Foundation

(WARF), Madison, Wisc. utilizing an amino acid analyzer and the automated ion-exchange method of Spackman et al. (1958).

The protein score for each essential amino acid was calculated (Pike and Brown, 1967) with the exception that only the methionine content rather than the total sulfur amino acids (methionine plus cystine) was taken into account due to the fact that no reliable data could be obtained for the cystine content of both the extracted and the original meal proteins. Whole egg was used as the reference protein.

*Biological evaluation.* For the biological evaluation, five isonitrogenous (by calculation) diets were prepared according to the AOAC (1965). The diets contained either the protein extract or the original coconut meal or casein or lactalbumin as the only protein source. Two diets were prepared with the protein extract, one containing the standard (AOAC) 1% crude fiber while an extra 3.85% crude fiber was added to the other diet in order to simulate the fiber content (4.85%) of the diet prepared with the untreated coconut meal.

Table 1—Percent composition of the original coconut meal and of the fiber free-coconut protein extract (dry basis)

	Protein (N X 6.25)	Crude fiber	Ash	Carbohydrates <sup>a</sup>	Ether extract
Coconut meal	21.90	11.85	5.05	47.35	13.85
Extracted product	31.80	0	8.62	43.73	15.85

<sup>a</sup> Carbohydrates by difference

Table 2—Percent composition and nitrogen content of the isonitrogenous diets evaluated

Ingredients	Diets <sup>a</sup>				
	CM	PE	PEF	Casein	Lactalbumin
Coconut meal	44.20				
Freeze dried extract		29.81	29.81		
Casein				10.70	
Lactalbumin					12.12
Cottonseed oil	2.35	3.43	3.43	8.00	8.00
Salt mixture <sup>b</sup>	2.93	2.51	2.51	5.00	5.00
Vitamin mixture <sup>b</sup>	1.00	1.00	1.00	1.00	1.00
Nonnutritive fiber <sup>c</sup>	4.85	1.00	4.85	1.00	1.00
Water	1.78	4.06	4.06	5.00	5.00
Sucrose	42.89	58.19	54.34	69.30	67.88
% Nitrogen	1.59	1.42	1.51	1.47	1.37

<sup>a</sup> Named after their protein source. CM = original coconut meal; PE = Protein (Coconut) extract; PEF = Protein (Coconut) extract plus 3.85% extra fiber.

<sup>b</sup> Same composition as that indicated in AOAC (1965) official method for the biological evaluation of protein quality according to which the diets were formulated.

<sup>c</sup> The 4.85% fiber content of the CM diet was that contributed by the coconut meal itself. No extra fiber was added to this diet.

<sup>1</sup> Present address: Institute of Nutrition of Central America and Panama (INCAP), P.O. Box 11-88, Guatemala City, Guatemala, Central America

**Table 3—Amino acid composition of the freeze-dried extract and of the original coconut meal (mg/g of material)**

Amino acid	Coconut meal <sup>b</sup>	Protein extract <sup>b</sup>
Alanine	7.59 (233)	12.30 (275)**
Ammonia	0.81 (25)	1.79 (37)
Aspartic acid	13.20 (404)	23.40 (488)
Cystine	N.C. <sup>c</sup>	N.C. <sup>c</sup>
Glutamic acid	31.00 (950)	55.00 (1146)
Glycine	5.95 (182)	11.30 (235)
Proline	4.70 (144)	8.32 (173)
Serine	6.84 (210)	14.10 (294)
Tyrosine	3.53 (108)	6.78 (141)
Arginine <sup>a</sup>	23.20 (711)	42.70 (890)
Histidine <sup>a</sup>	2.43 (74)	5.60 (117)
Isoleucine <sup>a</sup>	5.04 (154)	8.96 (187)
Leucine <sup>a</sup>	9.88 (303)	17.70 (369)
Lysine <sup>a</sup>	5.46 (167)	11.40 (238)
Methionine <sup>a</sup>	3.09 (95)	4.41 (92)
Phenylalanine <sup>a</sup>	6.00 (184)	11.10 (231)
Valine <sup>a</sup>	7.76 (238)	13.50 (281)
Threonine <sup>a</sup>	4.75 (146)	8.36 (174)
Tryptophan <sup>a</sup>	1.38 (42)	2.33 (49)

<sup>a</sup> Ten essential amino acids<sup>b</sup> Figures in brackets represent mg of amino acid per gram of N.<sup>c</sup> N.C. = not calculated.

A no-protein diet was also prepared having the same nutrients percentages with the exception of additional sucrose being substituted for the protein removed.

The carbohydrate source was sucrose (Domino sugar obtained locally). The salt mixture (U.S.P. XVII), vitamin mixture (AOAC), cottonseed oil, nonnutritive fiber (cellulose type) used in the preparation of the diets were obtained from General Biochemicals (Chagrin Falls, Ohio). The casein and lactalbumin used were also purchased from the same company. The proximate composition of the original coconut meal and the extracted material is given in Table 1. The percentage composition of the diets prepared and their nitrogen content are given in Table 2. All diets were mixed in a liquid-solid blender (Patterson-Kelley Co., East Stroudsburg, Pa.).

The animals used in this study were male, weanling (55–60g) Sprague-Dawley rats, 27 days of age for the net protein utilization

(NPU) and net protein retention (NPR) estimations and 40–50g, 21 days of age for the PER and apparent NPU evaluations. They were housed in individual stainless steel wire screen cages in a temperature controlled room. The lights were set for 12 hr of light and 12 hr of dark.

Ten animals per diet were used for the PER and apparent NPU tests and seven animals per diet were used for the NPU and NPR tests. A group of nine animals matched by weight with those used for the NPU and NPR tests were placed on the no-protein diet.

Diets and water were administered ad libitum. Food consumption and animal weights were recorded twice a week.

The PER was estimated following the method described by AOAC (1965). The NPR values were calculated according to Campbell (1963). The apparent NPU (or Productive Protein Value, PPV) values were estimated according to Cremer (1963) after an experimental

period of 28 days in five out of the ten animals used for the PER tests. The NPU values were determined according to Miller (1963).

## RESULTS & DISCUSSION

THE AMINO ACID composition of both, the freeze dried extract and the coconut meal is given in Table 3. The extract had a higher content (mg of amino acid per gram of extract) of every essential amino acid than the original coconut meal. The higher content of essential amino acids was also observed when the results were expressed as mg of amino acid per gram of nitrogen, except in the case of methionine which gave a slightly lower figure (92 mg/g of N) for the extract than for the coconut meal (95 mg/g of N).

The lysine content of the coconut meal (5.46 mg/g of material or 167 mg/g of N) indicates this to be the probable limiting amino acid, agreeing with the findings of Curtin (1958). However, the extract had a higher and more satisfactory lysine content (11.4 mg/g of extract or 238 mg/g of N) thus making it less probable that lysine is the limiting amino acid. The fact that the lysine content of the extracted proteins is much higher than that of the original coconut meal is of nutritional importance considering that the arginine content of both the extract (42.7 mg/g of extract or 890 mg/g of N) and the coconut meal (23.2 mg/g of material or 711 mg/g of N) is quite high. O'Dell and Regan (1963), Jones et al. (1966), O'Dell and Savage (1966) and Stutz et al. (1971) have clearly shown a lysine-arginine antagonism to occur in different animals. Further, it should be noted that although the arginine content of the extract was higher than that of the coconut meal, the increase was proportionately smaller than that obtained in the case of lysine. From a practical standpoint the high lysine content of the extract when compared to that of the coconut meal is also interesting, because

**Table 4—Protein score<sup>a</sup> of the coconut meal (CM) and coconut protein extract (PE)**

Material	ILe	Leu	Lys	Phe	Tyr	Met	Thr	Trp	Val	Total
Amino acid content (mg/g of N)										
PE	187	369	238	231	141	92	174	49	281	1762
CM	154	303	167	184	108	95	146	42	238	1437
Whole egg	415	553	403	365	262	197	317	100	454	3066
Percentage of total essential amino acids										
PE	10.61	20.94	13.50	13.11	8.00	5.22	9.88	2.78	15.95	
CM	10.72	21.09	11.62	12.80	7.52	6.61	10.16	2.92	16.56	
Whole egg	13.54	18.04	13.14	11.90	8.55	6.42	10.34	3.26	14.80	
Protein score for amino acid										
PE	78.36	100	100	100	93.56	81.31	95.55	85.27	100	
CM	79.17	100	88.43	100	87.95	100	98.26	89.57	100	

<sup>a</sup> Calculated according to Pike and Brown (1967)

it will allow the blending of the extract with other edible protein materials which may not necessarily have a high lysine content because this amino acid is commonly limiting in many plant proteins.

The protein score for each essential amino acid of both the freeze-dried coconut protein extract and the coconut meal is given in Table 4. The essential amino acid content and the percentage of total essential amino acids of the fiber-free coconut protein extract (freeze dried), coconut meal and whole egg (Pike and Brown, 1967) is included.

It is interesting to note that while the total essential amino acid content of the extracted proteins was higher than that of the coconut meal the percentage of such essential amino acids remains relatively similar. These results, however, indicate that a higher amount of essential amino acids would be supplied by the extract than by the coconut meal when both materials are considered on an isonitrogenous basis.

These results also indicate that isoleucine is the most limiting amino acid for both the extract and the coconut meal used, with a protein score of 78.36 and 79.17, respectively. It is also noteworthy that while tyrosine, threonine and tryptophan appear to be limiting in both cases, methionine is limiting only in the extract and lysine only in the coconut meal. In this respect it should be noted that the amino acid methodology for methionine (as well as tryptophan and cystine) tend to give lower values, thus the values of methionine in the extract and that of tryptophan for both extract and coconut meal could be higher than those reported here. However, the fact that lysine is limiting only in the coconut meal and not in the extract is quite evident. The protein extract appears to have, percentagewise, a higher lysine content than that of the whole egg reference standard.

Table 5 presents protein quality results for the protein extract, the original meal and the extract with 3.85% fiber added to simulate the fiber content of the original

meal diet. The protein quality results obtained for the control isonitrogenous diets (by calculation) prepared utilizing casein and lactalbumin are included.

It is evident that by all the methods used to assess protein quality, the protein extracted by the enzymic-chemical method (Molina and Lachance, 1973) was consistently superior to that of the original coconut meal, even when fiber equal to that of the original meal was added to the extracted product. The lower values when fiber was added illustrates the direct effect of the high fiber content of the coconut meal on protein nutritive value. These results are in accordance with the findings of Rama Rao et al. (1965) who reported that the biological value of the coconut proteins decreased considerably as the fiber content of the material increased.

The differences between the values obtained for the proteins of the extract and those of the coconut meal are, in all cases, statistically significant. In the case of the PER values the difference is statistically significant at a 95% confidence level ( $\alpha = 0.05$ ). Thus, the biological assays of protein quality clearly indicate that the protein is available and that the presence of fiber can compromise the final result even when the test diets have similar nitrogen contents.

Although the zero fiber content of the extract appears to be a definitive factor in enhancing biological value one cannot disregard the possibility that the change in the essential amino acid profile during the protein extraction could account, partly at least, for the improvement in nutritive value especially when considering that the extracted protein has a higher content of essential amino acids (mg/g of N) than the coconut meal proteins (see Table 4), and that the lysine-to-arginine ratio is relatively higher in the former (0.27) than in the latter (0.23).

The higher nitrogen intake observed in the animals on the coconut meal diet when compared to those on the extract diet could possibly be explained by a combination of two factors: (1) the

higher content of essential amino acids (mg/g of N) of the latter diet; and (2) the cathartic effect of the relatively higher fiber content of the former diet when compared to the latter diet. Although this second factor has been shown clearly to compromise the final results, little is known as to what extent the improved amino acid profile could affect the intake. The consistently improved protein biological values for the extract and the extract plus 3.85% fiber probably represent a benefit attributable to a more balanced amino acid profile.

Another possibility is that the enzymatically extracted protein, being partially broken down by the proteolytic enzyme (ficin), is more digestible than the protein in the original coconut meal.

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Table 5—Protein nutritive of the coconut protein extract compared to the original coconut meal, casein lactalbumin

	HSD <sup>a</sup>	No. of animals	CM <sup>b</sup>	PE <sup>c</sup>	PEF <sup>d</sup>	Casein	Lactalbumin
NPU	1.92	7	47 ± 0.65	56 ± 1.07	52 ± 0.85	69 ± 1.00	95 ± 1.41
NPR	0.60	7	3.81 ± 0.28	4.49 ± 0.36	3.94 ± 0.33	3.72 ± 0.32	6.71 ± 0.21
NPU app	2.97	5	47 ± 0.72	55 ± 1.51	49 ± 1.68	58 ± 0.75	—
PER	0.45	10	2.69 ± 0.10	3.09 ± 0.10	2.91 ± 0.17	2.86 ± 0.08	—
PER corrected			2.36	2.72	2.55	2.50	—

<sup>a</sup> "Honestly significant difference," calculated according to Tukey's w procedure ( $\alpha = 0.01$ ).

<sup>b</sup> CM = Original coconut meal.

<sup>c</sup> PE = Coconut protein extract.

<sup>d</sup> PEF = Extract plus 3.85% extra fiber.

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## USE OF ANIMAL BLOOD AND CHEESE WHEY IN BREAD: NUTRITIVE VALUE AND ACCEPTANCE

### INTRODUCTION

RECENT EMPHASIS upon pollution abatement has dictated that formerly accepted (or tolerated) food industry waste disposal practices be drastically curtailed (Ben-Gera and Kramer, 1969; Kerrigan, 1971). Relatively rapid progress has been made with cheese wheys and a range of practical recovery and utilization processes is evolving (USDA, 1970; 1972). Various baked goods, breading mixes, puddings, confections, beverages and ice cream can effectively contain whey (Webb, 1970).

In contrast, animal blood utilization appears more difficult and hence, less extensively practiced. For human use, blood processing schemes involve separation of the highly colored red cells from the plasma. The plasma is suitable for incorporation into meat emulsions (Gorden, 1971; Satterlee et al., 1973). The red cells may be relegated to animal feed (Pals, 1970). The protein from whole blood or plasma can be purified and concentrated by membrane techniques to produce a bland, light-colored, high-protein powder (Porter and Michaels, 1971). After hemolysis, the hemoglobin may be treated chemically to remove the heme group and spray-dried for food uses (Tybor et al., 1973). However, at present the principal outlets for animal blood are as animal feed ingredients and fertilizer. Over 95% of the 2 billion pounds of blood generated by the animal industries in the U.S. is recovered with an attendant reduction of 42% in waste effluent from packing plants (Garner et al., 1971). Only a negligible quantity presently is utilized as a human food ingredient.

The use of animal blood in human food products is common to many diverse cultures, but is not widespread in the U.S. Distinctive blood puddings and blood sausage products are well accepted in many European and Asian countries. Blood is consumed in the fresh state directly from cattle by some tribes in Africa (LaFoy, 1971).

Bread is an ideal nutrient-enrichment vehicle due to its use as a food staple in various forms throughout the world, its convenience of use, stability and high acceptance in low income diets. Some quite practical protein enrichment formulations and production techniques have been successfully developed (Kim and

DeRuiter, 1968; Tsen et al., 1971; Lorey and Maga, 1972).

The only encountered mention of utilizing blood in bread was in two German studies promoted by the food shortage during the first World War when it was recommended that 10% blood be added to all meals (Kobert, 1915). Apparently, the practice was not too successful, since a process was subsequently proposed which employed refrigeration for blood coagulation and serum separation, followed by the addition of hydrogen peroxide in order to mask blood in bread (Droste, 1915). In contrast, the use of whey or whey derivatives as substitutes for powdered milk in bread has been thoroughly explored and incorporation procedures established (Webb, 1970; Guy, 1972).

This study was designed to explore a simple method of utilizing unrefined blood and whey in bread and to establish the influence of these ingredients upon nutritive value and acceptance.

### EXPERIMENTAL

THE FORMULATION for pan bread and the blood or whey modifications was adapted from a standard recipe (Sultan, 1965) (Table 1). Whey was collected during vat drainage of a cottage cheese manufacturing operation on campus. Since whey contains a loaf depressant, it was heated to 85°C for 30 min (Sultan, 1965) and held at 2°C until used. Pork blood was collected as sanitarily as possible from stunned animals at the time of slaughter at a local commercial packing house. The blood was used fresh—within 1 or 2 hr after collection, or held refrigerated at 2°C for up to 2 days. It was necessary to comminute even freshly drawn blood in a blender in order to disperse the clot which formed within minutes after bleeding.

All-purpose flour, dextrose, salt and powdered milk (pan bread control only) were dry mixed. The liquid—water, whey or blood—was divided: 1/3 was mixed with the compressed yeast and shortening, and 2/3 was mixed with the dry ingredients in a Hobart 3-qt mixer. When the dough stiffened, the yeast mixture was added, and mixing continued until a smooth, elastic, well-developed dough formed (7–12 min). The dough was then partitioned by 500g portions into Teflon-lined baking pans, proofed for 1 hr at 37°C, 100% relative humidity, punched down, allowed to rise for 30 min, placed in a preheated electric oven and baked 20 min at 240°C. After cooling, the loaves were evaluated for specific volume, using rice as the displacement medium and prepared for analysis, panel evaluation or feeding studies.

Analyses (AOAC, 1970) consisted of moisture (vacuum oven, 70°C, 16 hr), lipid by petroleum ether soxhlet extraction for 6 hr, protein by micro-Kjeldahl (factor 6.25) and ash (4 hr at 600°C).

General acceptance was determined by an untrained panel of 10 persons who were not adversely affected by the concept of animal blood in food. In view of the vast differences in bread types, samples were grouped according to appearance as follows: (1) white pan, whey and commercial white breads; (2) an "organic" whole wheat from a local health food store, blood:whey 1:3 and commercial whole wheat; (3) blood bread, blood:whey 3:1, 1:1 and commercial pumpnickle.

16–24 hr after baking, samples were sliced 1.2 cm thick (corresponding to commercial reference samples) and 4 cm disks lacking crust or heel were cut from the slices. Each group was presented to panelists along with a pat of margarine. A 5-point modified multiple comparison scoring sheet was used with the reference sample assigned a general acceptance score of 3 (5 = much superior, 1 = much inferior). The tasting of each bread group was replicated twice.

Feeding studies were conducted with equally weighted groups of 10 male white rats,

Table 1—Bread formulations<sup>a</sup>

Type	NFDM	Cottage cheese whey	Pork blood	Shortening
Pan bread	2	—	—	—
Whey	—	32	—	—
Blood	—	—	32	2.5
Blood:whey, 3:1	—	8	24	2
Blood:whey, 1:1	—	16	16	1
Blood:whey, 1:3	—	24	8	0.5

<sup>a</sup> All doughs contain (parts by weight): dextrose 3, salt 1, compressed yeast 3, flour 54, shortening 2, fluid 32 (8 for yeast + 24). Adapted from Sultan (1965).

21–23 days old, housed in individual cages. Diets were adjusted to about 10% protein derived solely from the specific bread sample and supplemented to make them otherwise complete (AOAC, 1970), and a casein control group was included. Rats were fed ad libitum with weight gain and food consumption periodically recorded for 28 days. Protein efficiency ratios (PER's) were calculated from 4-wk weight gain/protein consumed.

Since the 90 weanlings were not available at one time, as litters achieved the proper age and weight, they were distributed randomly among the eight experimental and casein control diets with initial weight averages maintained within 0.5g. Thus, although each rat was on the diet 28 days, the total feeding period extended about 3 months.

Taste panel and feeding data were subjected to analysis of variance and means compared by a multiple range test (Duncan, 1955).

**RESULTS & DISCUSSION**

**BAKING PROPERTIES** of breads incorporating whey and/or blood in lieu of water were similar to the pan bread control. In all cases, specific bread loaf volumes were much lower and crumb texture much coarser and open-grained than commercial breads (Table 2, Figure 1). These defects are probably a reflection of the short proofing time and lack of baking expertise, since subsequent baking trials using extensive hand-kneading and two proofings produced a much

**Table 2—Specific loaf volume of experimental bread samples**

Bread type	Specific volume cc/g
Commercial white	5.66
“Organic” whole wheat	4.04
Commercial whole wheat	5.30
Pumpernickle	3.36
Pan control	3.38
Whey	3.08
Blood	2.85
Blood:whey, 3:1	3.10
Blood:whey, 1:1	3.03
Blood:whey, 1:3	3.13

improved blood bread loaf, but still inferior to commercial loaves. It is anticipated that proper use of the newer emulsifiers would further reduce these quality defects (Kim and DeRuiter, 1968; Tsen et al., 1971).

It was necessary to increase the amount of shortening in the blood containing formulations. Otherwise, the dough was excessively sticky and difficult to mix or handle. The whey bread was similar in appearance and taste to the pan bread control, while the blood bread and blood:whey mixtures were progressively

darker with increasing proportion of blood (Fig. 2). The 1:3 loaves were light brown, similar to whole wheat, whereas the 100% blood bread was as dark as commercial pumpernickle. The 100% blood bread also had an atypical glassy sheen in the crust and a slight cooked liver aroma when warm. After setting several hours, the liver aroma disappeared and was not evident when fresh slices were toasted.

Bread composition is shown in Table 3. Substituting whey for skim milk solids increased protein content slightly, whereas, both protein and lipid (partially due to increased shortening) increased with blood additive level. Blood bread contained about 1.7 times and “organic” bread 1.2 times the protein of white bread.

In the taste-panel evaluations of the various bread formulations, the experimental loaves were found generally comparable to the commercial reference samples (Table 4). Panelists were screened to eliminate persons with objectional attitudes toward consuming blood; thus the panels consisted of persons with a more tolerant and experimental attitude toward food than the population at large. An unbiased (uninformed) panel would have been preferred experimentally, but not ethically.

The “organic” bakers were capable of producing a better-appearing loaf, although at 1.4 times the price of whole wheat bread. But the acceptance of this premium bread was not significantly different from the whole wheat reference or the blood:whey 1:3 mix.

The feeding study with the breads supplying about 10% protein to otherwise adequate diets is reported in Table 5. The organic whole wheat bread was not signif-

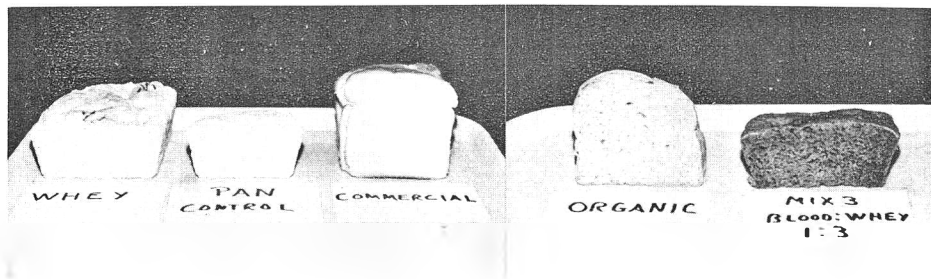


Fig. 1—A comparison of experimental, organic and commercial white breads.

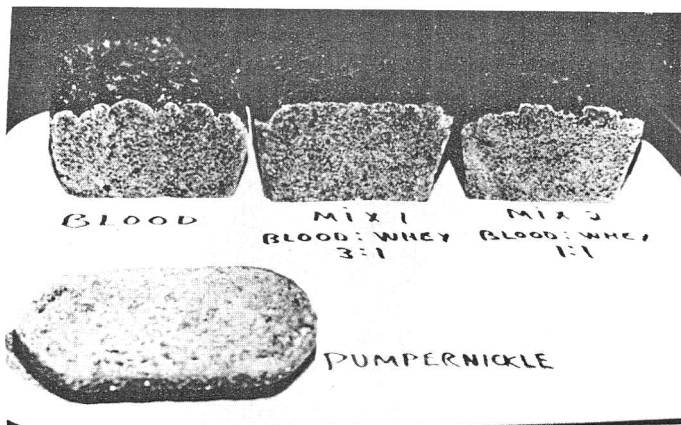


Fig. 2—A comparison of blood:whey breads and commercial pumpernickle.

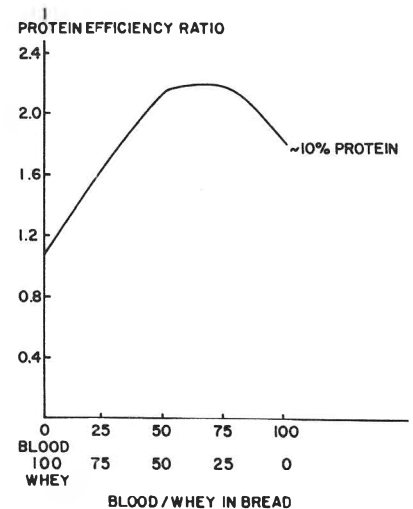


Fig. 3—Effect of blood:whey ratios on protein efficiency ratios of breads.



Table 3—Bread composition

Type	Protein	Fat	Ash	Moisture
	(% Dry weight basis)			(as baked)
Commercial white	11.5	2.5	2.4	37.6
Pan control	11.6	2.8	2.8	39.0
"Organic" whole wheat	14.2	1.8	2.6	32.6
Whey	12.9	3.3	2.7	33.4
Blood	19.2	6.7	2.4	33.6
Blood:whey, 3:1	17.6	5.3	2.4	32.0
Blood:whey, 1:1	14.2	4.5	2.6	33.3
Blood:whey, 1:3	12.6	3.7	2.5	34.3

Table 5—Protein efficiency ratios of bread diets<sup>a</sup>

Diet	Weight gain g	Protein consumed g	PER	Adjusted PER <sup>b</sup>
Commercial white	12.3	16.5	0.75	0.64a
"Organic" whole wheat	14.0	15.9	0.88	0.76ab
Pan control	15.0	15.4	0.98	0.83bc
Whey	18.3	17.6	1.04	0.90c
Pork blood	33.3	18.4	1.79	1.52d
Blood:whey, 3:1	48.5	22.3	2.19	1.85e
Blood:whey, 1:1	60.6	28.1	2.15	1.82e
Blood:whey, 1:3	32.9	20.3	1.63	1.38d
Casein	66.2	21.5	2.96	2.50f

<sup>a</sup> Average of 10 rats/diet, 28-day feeding @ 10% protein

<sup>b</sup> Means followed by the same letter are not significantly different at the 0.05 level.

icantly better than commercial white bread. Whey provided some improvement and blood bread had a fairly acceptable PER of 1.52. The PER's achieved with the blood:whey 1:1 and 3:1 breads, 1.82 and 1.85 respectively, are comparable to that obtained with 12% soy flour 1.83 (Kim and DeRuiter, 1968). Figure 3 contrasts the feeding studies with blood:whey compositions. A small amount of whey, or in its absence, perhaps powdered milk or soy flour, can have a quite beneficial influence upon breads incorporating animal blood in lieu of water in the baking formulation.

Nationwide publicity and vocal claims to the contrary, the "organic" whole wheat bread was not significantly different in protein value from any of the other commercial or experimental breads evaluated. Blood, whey or judicious mixtures of protein supplements appear to be much more effective improvers of bread-based nourishment than the "organic mystique."

The addition of unrefined animal blood to bread or other foods in the U.S. is probably impractical on aesthetic grounds, although refined, colorless bland blood derivatives may eventually be commercially available (Pals, 1970; Porter and Michaels, 1972). In cultures with a less negative attitude toward animal blood and greater need for high quality protein, simple utilization of sanitary, unrefined blood may be practical, and

an attractive alternative to allowing the blood to become a pollutant. In situations where baking is performed in close proximity to animal slaughter operations and the respective production schedules are compatible, a valuable outlet for blood is possible—provided that rapid, sanitary handling and incorporation of the blood is practiced.

During baking, the blood is essentially pasteurized, dehydrated and stabilized—processes frequently considered too complex and costly to perform on a small scale, but readily accomplished as part of the baking process. Process refinements by which some blood decolorization is accomplished might further enhance the practicality of blood utilization, provided that the economics were not adversely affected.

Whereas whey as a substitute for water in baking has a slight beneficial influence upon bread nutritive value, it is particularly effective when combined with blood. However, both ingredients are relatively dilute solutions, subject to rapid spoilage and must be stabilized or processed rapidly. Traditionally, whey and blood are serious pollutants. The traditional baking process appears to be a practical means of transforming them into valuable food resources.

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Table 4—Acceptance of bread samples

Group	Score <sup>a</sup>
I	
Commercial white	3.0a
Pan control	3.9b
Whey	3.8b
II	
Commercial whole wheat	3.0c
"Organic" whole wheat	2.9c
Blood:whey, 1:3	3.2c
III	
Commercial pumpernickle	3.0d
Blood	2.6d
Blood:whey, 3:1	2.8d
Blood:whey, 1:1	2.7d

<sup>a</sup> Means within the same group followed by the same letter are not significantly different at the 0.05 level.

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## BONE PARTICLE CONTENT OF SOME MINCED FISH MUSCLE PRODUCTS

### INTRODUCTION

CONVENTIONAL FISH blocks are made from whole fish fillets. In recent years, because of the diminishing supply of and increasing demand for species usually used for processing into blocks, prices for this product have risen sharply. The economics of supply and demand have made it necessary to develop methods for the production of blocks from the flesh of less expensive species and possibly from the flesh left on the trimmings and frames (the skeletal structure and adhering flesh remaining after removal of fillets) of the various species used for fillets and steaks. The use of these "new" sources of fish muscle has been made practical by the development of machines that efficiently separate edible flesh from skin and bones. Such machines are manufactured in the United States (Paoli Intl., Rockford, Ill. and Beehive Machinery, Salt Lake City, Utah), Japan (Bibun Kikai Seisakusho, Hiroshima-ken; Ikeuchi Takko Sho, Akeishi; and Yanagiya Machinery, Yamaguchi Prefecture), Sweden (Iwema, Goeteborg), and Germany (Baader, Lubeck). Commercial fish blocks are now being produced in relatively small amounts from minced flesh separated by these machines. The minced muscle turned out by these machines may contain amounts of bone. The frequency of occurrence of bones (fragments) depends largely on the size of the final extrusion opening through which the flesh is passed. Short fragments appear in the minced flesh when the bones are aligned transversely across the extrusion openings and are sheared off and pressed through with the flesh. Longer fragments appear when, by chance, they are aligned axially with the extrusion openings and are pressed part way through before being sheared off. Bone fragments may be soft and pliable and not easily detected during eating of the cooked product. They may also be sharp-pointed and rigid and potentially harmful. A potentially harmful bone is considered to be one that leaves a clear indentation when it is pressed axially between the fingers. Such bones should be eliminated from the product.

Recent publications on the use of machine-separated minced muscle have not considered bone content (Crawford et al., 1972; Teeny and Miyauchi, 1972; Miyauchi and Steinberg, 1970; King and

Carver, 1970). Patashnik et al. (1973) indicate the presence of "a few small bone particles" in the machine-separated minced flesh but did not experience any problems in using it in the several finely emulsified product types they evaluated. However, in fish sticks and portions, a significant effect on consumer acceptance may be anticipated inasmuch as bones can usually be detected more readily.

At a Technical Seminar on mechanical recovery and utilization of fish flesh held in Oakbrook, Ill. in 1972, there was a surprising lack of information on the bone content of machine-separated minced flesh. Representatives from industry expressed the need for this information as well as for information on other quality attributes of machine-minced flesh. They also made the point that orderly marketing will require the establishment of standard nomenclature and quality criteria for both the raw material and the minced-fish end product. To help assure that these products find their place in the U.S. market, the National Marine Fisheries Service is developing a "Proposed United States Specification for Minced Fish Blocks." This document is intended to answer the immediate marketing needs of industry and to function on an interim basis until a Federal Grade Standard can be developed and promulgated.

To provide data useful in the preparation of the proposed specification, a series of experiments were carried out to (1) develop an objective procedure for determining the bone particle content of minced fish flesh; (2) determine the bone particle content of minced fish flesh produced by some flesh-separating equipment using different types of raw material; and (3) determine the bone particle content of some commercial samples and its possible effect on consumer acceptability. The results are reported here.

### EXPERIMENTAL

THE BONE PARTICLE content of minced fish flesh produced under different conditions was determined using a gravity-flotation method. The ease of sensory detection of these particles and their effect on product acceptance by a taste panel was also determined.

#### Samples of minced fish blocks for bone particle analysis

**Experimental.** Minced rockfish flesh was

obtained from three forms of raw materials: (1) washed, headed-and-gutted (H&G) black rockfish (*Sebastes flavidus* and *S. melanops*); (2) skin-on fillets including vee-bones cut from H&G black rockfish; and (3) the remaining separated frames. The raw materials were passed through a commercial-size Bibun Flesh Separator equipped with a rotating drum perforated with 7-mm diameter holes. Minced "lox" flesh was obtained by passing salt-cured, smoked salmon trimmings through a laboratory-size Yanagiya Flesh Separator "Miny" model (Miyauchi and Steinberg, 1970) equipped with a perforated rotating drum with 4-mm diameter holes. Part of the minced flesh of both species was then passed through a Bibun Flesh Strainer (King and Carver, 1970) equipped with 1-mm or 2-mm holes to further debone the flesh which was then frozen into blocks.

For sensory evaluation, minced black rockfish blocks were prepared by the method of Teeny and Miyauchi (1972).

**Commercial.** Samples of commercially prepared frozen minced blocks were taken at random off the processing line of a fish-stick plant. The numbered slab samples in Table 5 were taken sequentially from a single block. Partial slab samples (3-oz subsections of a slab) and unbreaded fish stick samples (0.5-0.6 oz) were taken at random off the production line during a 15-min period. At the same time, battered-and-breaded precooked fish stick samples (1-oz) were taken for sensory evaluation.

#### Sensory detection of bones

**Experimental samples.** Frozen blocks were sawed into 1-1/8 in. × 1-1/2 in. × 5/8 in. thick (1-1/2 oz) portions. They were battered and breaded in the frozen state after which they were deep-fat fried at 177°C for 5 min. The sensory panel was instructed to note the number and sizes of the bone particles found.

**Commercial samples.** The precooked battered-and-breaded fish sticks were heated in an oven at 204.5°C for 15 min. The sensory panel was instructed to chew completely each sample and to rate the sample for acceptability on the basis of the number and size of bone particles detected.

#### Gravity-flotation method for determining bone content

The method involves two steps: (1) shredding the flesh in water with a low-speed stirring device and (2) gravity separation of the bone, cartilage and other high-density components from the lower-density floatable muscle fibers.

**Equipment.** (1) 8-speed food stirrer-blender-disintegrator (Oster Model 847 or equivalent)—the cutting edges of the blades are blunted or rounded; (2) 5-cup blender jar; and (3) paper towels.

**Procedure.** (1) Weigh 50 or 100g of fish sample, which may be either frozen or unfro-

zen; (2) place sample in 5-cup blender jar and fill with cold tap water; (3) blend at lowest speed for about 2 min; (4) place blender jar in sink and run tap water into it at a flow rate of about 1 gal/min until water is essentially free of floating fish muscle fibers, break up any remaining aggregates of flesh at the bottom of the jar or on cutter blade, and float off fibers with running water; (5) transfer quantitatively the bone, fin and cartilage residue to a paper towel for estimating or counting; (6) for counting, separate into components (bones, cartilage, skin pieces, etc.); and (7) sort bones into various size categories (< 1/8 in., 1/8 in. to 1/4 in., 1/4 in. to 1/2 in., > 1/2 in.), count and report as number per pound of sample: Number of bone particles per pound =  $(454.4/\text{sample wt}) \times \text{bone count}$ .

The above size categories and method of reporting bone particles were selected mainly for potential use in preparing the "Proposec United States Specification for Minced Fish Blocks." In practice, the method of reporting bone particles and other nonmuscle components will depend upon product specification requirements or the type of quality information needed by the buyer or seller of the products.

To determine the reproducibility of the procedure and to check that bones are not being reduced in size during blending, the following test is suggested: (1) add 10-20 bones of known size to a bone-free sample of minced flesh; (2) run through the above procedure to verify that all bones can be recovered and that there is no bone fragmentation; (3) repeat as required to assure reproducibility of the procedure and that the cutting edges of the blender blades have been adequately blunted or rounded.

## RESULTS & DISCUSSION

### Bone particle content of experimental samples

By gravity-flotation method. Tables 1-3 show the bone content of various experimental samples put through the flesh separator and separator/strainer. The data in Table 1 compare the average number of bones for each size category per pound of minced flesh after passing headed-and-gutted rockfish through the Bibun flesh separator (7-mm perforations) under minimum pressure and after passing the resultant residue through the separator again but under medium pressure. The total bone particles per pound for the first-pass flesh was 24 and for the second-pass flesh was 127. By putting portions of the first-pass minced flesh through either the 2-mm or 1-mm strainers, the total bone particle contents were respectively reduced to 6.5 and 0.1 per pound; similarly by straining the second-pass flesh, bone particle content was reduced to 38 and 3 per pound, respectively. The 2-mm strainer reduced the bone particle content of the first- and second-pass flesh by 70-73%. The 1-mm strainer achieved 98-100% reduction for both. Due to the high initial bone particle content of the second-pass flesh, the 1-mm strainer is needed to effectively remove the bones from this material.

On passing "lox" belly trim (salt-cured smoked salmon) through the Yanagiya flesh separator (4-mm perforations) at minimum pressure, the first-pass flesh contained 32 bone particles per pound; second-pass flesh contained 116 bone particles per pound (Table 2). When the first-pass flesh was put through the 2-mm strainer, the bone particle content was reduced by 94% to 3 particles per pound. Second-pass material was reduced by only 72% to 32 particles per pound. Here too, because of the high initial bone particle content of the second-pass material, the need for a 1-mm strainer is indicated.

The results of experiments to compare the bone particle contents of first-pass Bibun-separated flesh from H&G rockfish, skin-on fillets (H&G rockfish with frames removed), and from frames alone are given in Table 3. As was expected, flesh from the H&G rockfish containing intact frames and flesh from the frames alone contained more bone particles than did the flesh from the skin-on fillets. The flesh from the H&G fish contained more bone particles than did the flesh from the frames-24.4 per pound against 17.1 per pound. This somewhat higher total bone particles content may be explained on the

Table 1—Average number of bones per pound of minced flesh from headed-and-gutted black rockfish passed through the Bibun flesh separator (7-mm perforations) and strainer (1- and 2-mm)

Treatment			No. of 50-g samples analyzed	Avg number of bones, by size, per pound of flesh			
				< 1/8"	1/8-1/2"	> 1/2"	Total
1st pass (minimum pressure)	Unstrained	(7 mm)	14	9.0	14.0	1.0	24.0
	Strained	(2 mm)	40	6.0	0.5	0.0	6.5
	Strained	(1 mm)	39	0.1	0.0	0.0	0.1
2nd pass (medium pressure)	Unstrained	(7 mm)	12	55.0	67.0	5.0	127.0
	Strained	(2 mm)	20	35.0	3.0	0.0	38.0
	Strained	(1 mm)	19	3.0	0.0	0.0	3.0

Table 2—Average number of bones per pound of minced "lox" (salt-cured smoked salmon) from trimmings passed through the Yanagiya separator (4-mm perforations) and 2-mm strainer

Treatment			No. of 50-g samples analyzed	Avg number of bones, by size, per pound of flesh			
				< 1/8"	1/8-1/2"	> 1/2"	Total
1st pass (light pressure)	Unstrained	(4 mm)	6	17	13	2	32
	Strained	(2 mm)	6	0	3	0	3
2nd pass (light pressure)	Unstrained	(4 mm)	6	53	53	10	116
	Strained	(2 mm)	6	30	2	0	32

Table 3—Average number of bones per pound of minced flesh from intact headed-and-gutted (H&G) black rockfish, from skin-on fillets (H&G rockfish with frames removed) and from frames passed through separator (7-mm perforations) at minimum pressure

Treatment	No. of 100-g samples analyzed	Avg number of bones, by size, per pound of flesh			
		< 1/8"	1/8-1/2"	> 1/2"	Total
Headed-and-gutted rockfish	43	12.0	11.5	0.9	24.4
Skin-on fillets (with vee-bones and belly wall left on)	30	2.0	1.3	0.0	3.3
Frames (central vertebral bone with tail and adhering flesh)	35	10.3	6.5	0.3	17.1

basis that more drum pressure is generated during the passage of the H&G fish than during the passage of the considerably thinner frame.

By sensory detection. Table 4 shows the test results of the sensitivity of an experienced sensory panel to the presence of bone particles in some of the experimental samples and compares this with the gravity-flotation method. The sensory

panel selected consisted of regular panelists each with 5 or more years of experience in the sensory evaluation of minced flesh products, such that they would be expected to be more sensitive to the presence of bones than the ordinary consumer. Using the flotation method on the first-pass H&G rockfish, we found 24.8 bone particles per pound of minced flesh. The sensory panel found only 1.7 bone

particles per pound or about 7% of those present. In the minced flesh from skin-on fillets containing 6.0 bone particles per pound by the flotation method, the sensory panel found only 0.6 bone particles per pound or about 10% of those present.

The wide difference in results obtained by the sensory panel and that of the objective method is largely due to the differing sensitivities of the two methods.

**Table 4—Average number of bones per pound of minced flesh from headed-and-gutted black rockfish and from skin-on fillets after passing through Bibun flesh separator (7-mm perforations). Determined by gravity flotation and by sensory panel<sup>a</sup>**

Treatment	Method of analysis	Samples analyzed		Number of bones, by size, per pound of flesh				Total
		Number	Size	< 1/8"	1/8–1/4"	1/4–1/2"	> 1/2"	
Headed-and-gutted whole rockfish (1st pass, minimum pressure, unstrained)	Gravity-flotation	36	100 g	15.0	5.0	4.0	0.8	24.8
	Sensory panel	392	1-1/2 oz	0.8	0.4	0.3	0.2	1.7
Skin-on fillets with vee bones & belly wall on (1st pass, minimum pressure, unstrained)	Gravity-flotation	15	100 g	4.0	2.0	0.0	0.0	6.0
	Sensory panel	24	1-1/2 oz	0.0	0.0	0.3	0.3	0.6

<sup>a</sup> An experienced panel consisting of our technical staff was used.

**Table 5—Average number of bones, by size, per pound of minced flesh from frozen blocks commercially prepared from fish of various species and imported from various countries**

Species used, block size, and country of origin	Sample source or description	No. of 100-g samples analyzed	Avg number of bones, by size, per pound of minced flesh <sup>a</sup>				Total
			< 1/8"	1/8–1/4"	1/4–1/2"	> 1/2"	
Atlantic pollock (chopped), 13-1/2 lb, Norway	Slab no. 1	5	59.0	33.0	11.0	3.0	106.0
	Slab no. 2	6	17.0	33.0	1.0	2.0	53.0
	Slab no. 3	6	11.0	11.0	5.0	2.0	29.0
		17	27.0	25.0	5.3	2.3	59.6
Cod (minced), 13-1/2 lb, Canada	Slab no. 1 & 2	9	1.5	0.5	0.0	0.0	2.0
	Slab no. 3 & 4	11	1.0	0.0	0.0	0.0	1.0
	Partial slabs	8	6.0	4.0	0.0	0.0	10.0
	Fish sticks	9	4.0	0.5	0.0	0.0	4.5
	37	4.0	1.5	0.0	0.0	5.5	
Cod (minced), 13-1/2 lb, Denmark	Slabs no. 1 & 2	8	254.0	147.0	65.0	0.0	466.0
	Slabs no. 3 & 4	6	294.0	33.0	27.0	0.0	354.0
	Slabs no. 5 & 6	8	267.0	105.0	0.6	0.0	372.6
	Fish sticks	17	330.0	162.0	75.0	1.4	568.4
	39	296.0	127.0	40.0	0.3	463.3	
Cod, 18-1/2 lb, Iceland	Slab no. 1	5	3.0	0.0	0.0	0.0	3.0
	Slab no. 2	5	0.0	0.0	0.0	0.0	0.0
	Fish sticks	10	6.0	0.0	0.0	0.0	6.0
		20	4.0	0.0	0.0	0.0	4.0
Alaska pollock, 18-1/2 lb, Japan	Slab no. 1	5	35.0	13.0	0.0	0.0	46.0
	Slab no. 2	3	68.0	8.0	0.0	0.0	76.0
	Slab no. 3	5	52.0	6.0	0.0	0.0	58.0
	Slab no. 4	5	51.0	16.0	0.0	0.0	67.0
	Slab no. 5	5	43.0	9.0	0.0	0.0	52.0
	Slab no. 6	5	45.0	4.0	0.0	0.0	49.0
	28	46.0	10.0	0.0	0.0	56.0	

<sup>a</sup> Figures shown beneath the broken line for each group of samples indicate the total number of samples analyzed and the average number of bones in the group. Averages were prorated on the basis of sample weight.

Table 6—Objective<sup>a</sup> and sensory<sup>b</sup> evaluation for the presence of bones and their effect on product acceptability of commercial samples of precooked fish sticks made from imported fish blocks

Species used, block size, and country of origin	No. of 100-g samples analyzed	Evaluation by gravity flotation <sup>a</sup>				Evaluation by sensory panel <sup>b</sup>		
		Avg number of bones, by size, per pound of minced flesh				No. of samples analyzed	Percentage of total number of samples	
		< 1/8"	1/8–1/4"	1/4–1/2"	> 1/2"		In which bones were detected	Rated unaccept- able
Cod (minced), 13-1/2 lb, Canada	37	3.0	1.0	0.0	0.0	24	0.0	0.0
Cod (minced), 13-1/2 lb, Denmark	39	296.0	127.0	40.0	0.3	24	29.4	4.2
Cod, 18-1/2 lb, Iceland	20	4.0	0.0	0.0	0.0	24	0.0	0.0

<sup>a</sup> Average number of bones per pound was objectively determined by the gravity-flotation method.

<sup>b</sup> The 24-member sensory panel was of consumer type made up of students and technical and office employees.

The sensory method involves a lower statistical probability that a bone of detectable size and hardness will be contacted during chewing. Also, small diameter or pliable bones, even when contacted during chewing, are difficult to sense by tactile means. In contrast, the objective method based on specific gravity, is independent of the size or hardness of bones for their reliability of detection.

#### Bone particle content of commercial minced fish blocks

By gravity-flotation method. Bone particles in minced commercial blocks were determined by the gravity-flotation method and are reported in Table 5. The data show that the bone particle counts per pound of product vary widely and are surprisingly high in some of the samples. They range from an average of 4 per pound for Icelandic cod blocks to an average of 463 per pound for cod blocks processed in Denmark. The Norwegian-processed Atlantic pollock blocks and the Japanese-processed Alaska pollock blocks were intermediate in bone particle content, 60 and 56 per pound, respectively. It should be pointed out that bone particle content appears to be a function of processing methods and raw material, not of species. All cod blocks were made of the same species, *Gadus morhua*. Atlantic pollock (*Pollachius virens*) is a distinctly different species from Alaska pollock (*Theragra chalcogramma*).

By sensory detection. The ability of a nonexpert or consumer-type sensory panel to detect the presence of bone

particles in some of the commercial samples was evaluated. The data in Table 6 indicate that only a small fraction of the bone particles actually present could be detected by the panel members even though they had been instructed to chew completely each sample in order to quantify presence of bones. Panelists did not find any bones in the Canadian and Icelandic cod, i.e., those virtually free of bone particles. However, in the cod blocks containing 463 bone particles per pound, 7 or 29.4% of the panelists detected bones, but only 1 or 4.2% found the product unacceptable because of the bone particles. The granular, crusty nature of the cooked batter and breading combined with the chewy fish flesh apparently conceals the presence of soft pliable bone particles.

Reduction of bone content. Although bone particles are not readily detectable by sensory means, their presence should be reduced to as low a level as feasible.

Straining the minced flesh has been shown to be an effective means of reducing the bone particle content to acceptable limits. However, straining reduces particle size of the flesh and may alter the texture of the cooked product. It probably has an effect on frozen storage characteristics and therefore on acceptability. Straining also increases product handling and production costs. The degree to which straining may be necessary to reduce the bone content will have to be carefully worked out in terms of product end use and the effect on product quality.

#### Use of gravity-flotation method as a quality control tool

The gravity-flotation method is recommended as a useful quality control tool to provide reasonably accurate estimates of the bone content. Depending on the accuracy desired, a test may take from a few minutes to 10 min per sample. Where only an estimate of the type and quantity of bones, scales and bits of skin and fins is needed for quality control screening, the procedure may be speeded up and the amount of extraneous material is visually estimated rather than counted.

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Trade names referred to in this publication do not imply endorsement of commercial products.

## SQUID PROTEIN ISOLATE: EFFECT OF PROCESSING CONDITIONS ON RECOVERY YIELDS

### INTRODUCTION

PROTEIN CONCENTRATES from marine animals have great potential as an additional source of protein to augment traditional protein supplies. They have a high nutritive value, and if an economical process producing concentrates with good functional properties can be developed, they should be valuable substitutes for the more conventional proteins.

Extensive reviews by Finch (1970) and Meade (1971) indicate that commercial efforts to make fish protein concentrate (FPC) for human consumption have largely concentrated on solvent extraction processes which employ isopropyl alcohol (IPA), ethylene dichloride, and, to a lesser extent, ethanol and hexane as extracting solvents. In general, these processes result in a protein with good nutritional properties, little or no offensive odor and taste, and no functional properties. Although much effort has been expended to use FPC in food systems, it appears that FPC without functional properties is usually reduced to the role of a food supplement.

Recently, consideration has been given to the use of fish for preparing functional protein isolates. Tannenbaum et al. (1970) reported that, in their investigation of FPC as a starting material for the preparation of protein isolates, FPC was partially hydrolyzed with sodium hydroxide and recovered as an isoelectric protein.

Meinke et al. (1972) discussed some of the factors that influence the production of protein isolates from fish, presenting data on the solubility characteristics and the recovery of isolates from whole raw fish and frozen fish at various pH's, salt concentrations and temperatures.

Spinelli et al. (1972) presented work on the separation of the basic protein components of fish muscle into the two major protein fractions (myofibrillar and sarcoplasmic). They also evaluated the use of some proteolytic enzymes to modify physical and chemical characteristics of the separated protein fractions.

Squid also has the potential for use as

a raw material to produce a soluble and functional protein concentrate. The squid is widely distributed, very abundant (Voss, 1973), palatable and nutritious. It has a low fat and high protein content. Most importantly, it contains a high proportion of water soluble protein: about 77–85% of its total protein content (Matsumoto, 1958).

The nutritional value of squid has been studied by Varela et al. (1926). The biological value (b.v.) of squid protein was reported to be 82 as compared to 83.7 for octopus, and 74.8 for shrimp.

A feasibility study on the preparation of squid protein concentrate by spray dehydration was carried out by Lee (1970). The parameters required for efficient processing, the yields that can be expected from the process and the properties of the concentrate produced were evaluated.

This work is a detailed investigation of the influence of various process parameters on the extraction and recovery of protein from *Loligo* species of squid.

Several process variables may be expected to influence the rate and extent of extraction, and the quality of the extracted protein. These include (1) concentration and particle size of suspended tissue; (2) extraction time; (3) pH of the extracting solution; (4) type and concentration of salts employed in extraction; and (5) extraction temperature.

### EXPERIMENTAL

#### Raw material: Procurement and preparation

Squid of Rhode Island origin were procured for experimentation. On arrival at the laboratory the squid were (a) sorted to remove non-*Loligo* species present; (b) packed tightly in 5-lb packages in polyethylene freezer bags (3–4 mils thick); (c) placed in a Haskris (Haskris Co., Chicago, Ill.) blast freezer at  $-40^{\circ}\text{C}$  and frozen for subsequent experimentation; and (d) transferred to a  $-40^{\circ}\text{C}$  frozen storage room for storage.

A typical proximate analysis of squid tissue is given below:

Water	80%
Protein	17%
Lipids	1.5%
Ash	1.3%

Prior to extraction, the squid were thawed overnight in their polyethylene bags under refrigeration (15 hr), and were then cleaned,

eviscerated and skinned. The cleaned mantles were ground in an Osterizer Model 480 Food Grinder, using plates with different perforation diameters. The samples were then processed according to the procedures outlined below.

#### Extraction

Ground squid tissue was mixed with the extraction medium for a measured length of time, at a constant temperature. The slurry was mixed in a beaker using a magnetic stirrer. The speed of rotation was adjusted so as to keep the squid tissue in suspension, without excessive foaming.

The slurry was prepared by adding the squid tissue to the aqueous solution containing the acid or alkali. The quantity of acid or alkali necessary to give the desired initial pH when mixed with squid was determined by trial runs. The pH was then kept constant during extraction by addition of dilute NaOH or HCl, as needed.

Following extraction, the slurry was centrifuged at  $3,000 \times G$  for 10 min. The supernatant was decanted through a filter of glass wool and analyzed for nitrogen.

The extraction variables were: particle size (2.38–8 mm); pH (2.5–11.0); type and concentration of extraction medium (sodium chloride, sodium trimetaphosphate, sodium hexametaphosphate at concentrations of 1–5%); extractant-to-squid ratio (20:1–3.3:1); temperature ( $5-60^{\circ}\text{C}$ ); and time (15–60 min).

#### Isoelectric precipitation

Protein from alkaline extraction (pH 11, sodium hydroxide) was precipitated by lowering the pH through the use of different acids (hydrochloric acid, sulfuric acid, phosphoric acid, acetic acid); it was recovered by centrifugation at  $3,000 \times G$  for 10 min. The supernatant extract was decanted through a filter of glass wool and analyzed for protein.

#### Analytical procedures

Nitrogen was determined by the micro-Kjeldahl method (AOAC, 1970).

Extraction yields (% of total nitrogen in solution) were computed as follows:

$$\frac{\text{g N in total vol of extract}}{\text{g N in squid sample}} \times 100 = \frac{\text{extraction}}{\text{yield}}$$

### RESULTS

#### Effect of tissue disintegration on extraction of protein from squid

Figure 1 shows the effect of tissue disintegration on protein extraction yields. The percent of protein extracted ( $P \leq 0.05$ ) significantly increased with each

<sup>1</sup> Permanent address: Dept. of Food & Biotechnology, Technion, Haifa, Israel

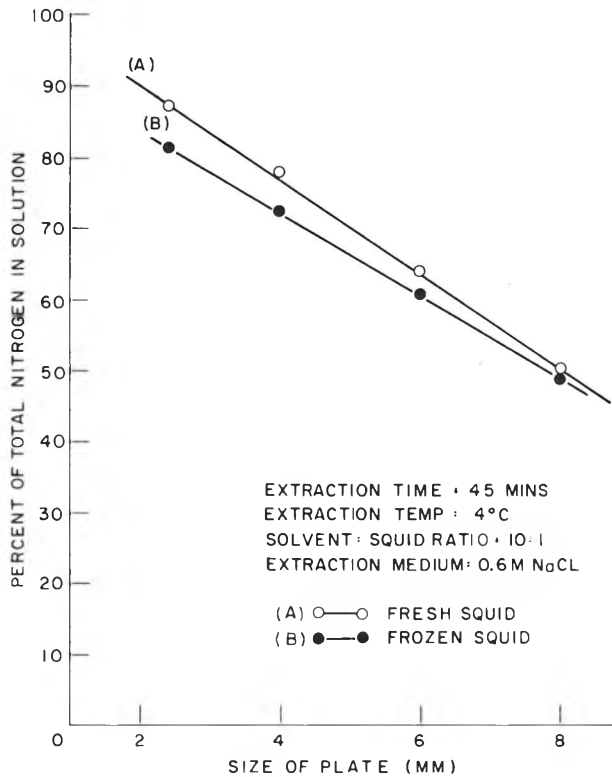


Fig. 1—Effect of particle size on protein extracted from squid.

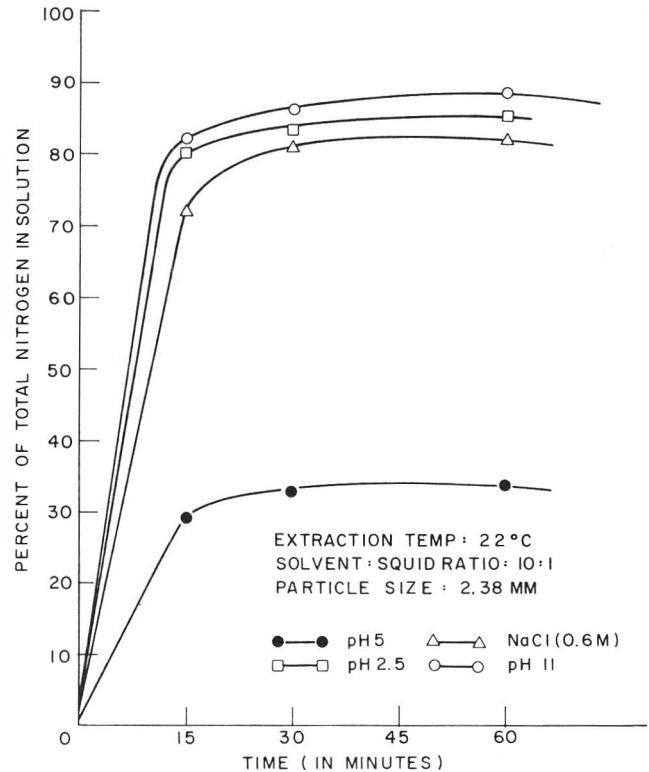


Fig. 2—Effect of extraction time on the amount of protein extracted.

step of tissue destruction. Therefore, the finest degree of grinding (2.38 mm plate) was adopted for all subsequent work.

#### Effect of freezing-thawing on the extractibility of protein

Line A in Figure 1 refers to iced fresh squid. Line B shows the extraction yields obtained on squid which had been frozen, stored for 1 wk at  $-40^{\circ}\text{C}$ , and thawed as described above. It is evident that freezing and thawing decreased protein solubility only slightly. In subsequent experiments, frozen squid was used as the raw material for convenience.

#### Effect of extraction time on the amount of protein extracted

The duration of extraction influenced the amount of protein obtained; this was investigated with a 0.6M NaCl solution as the extractant and in aqueous solution at pH 2.5, 5.0 and 11.0. The results, which are presented in Figure 2, show that the extraction of squid protein occurs fairly rapidly, with little further protein extraction occurring after 30 min. All subsequent extraction trials were carried out for 45 min.

#### Effect of pH on the solubility of protein

The protein solubility profile (Fig. 3)

indicates minimum protein solubility within pH range 4.5–5.5. Within this pH span only 32–35% of squid protein goes into solution. On both the acid and basic

sides of this pH range the protein solubility increases, reaching 81% at pH 2.5 and 88% at pH 11.0. In these experiments pH was adjusted using NaOH or HCl.

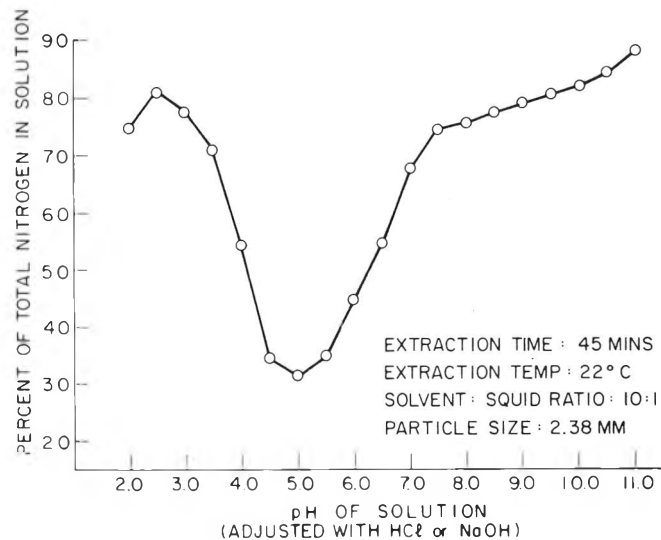


Fig. 3—pH-solubility profile.

**Effect of the ratio of weight of squid to volume of extracting medium**

The data (Fig. 4) show that the extraction yields increase with increasing

extractant-to-tissue ratios. The use of high extractant-to-tissue ratios results in very dilute extracts which would be more costly to handle. On the other hand, low ratios of extractant to squid, while yielding more concentrated protein extracts,

produce problems of viscosity and gelation. It is also logical to assume that less extract can be recovered from the slurry as the weight of the squid relative to the aqueous extractant increases. Thus the selection of an extractant-to-tissue ratio is a compromise between recovery yield and extract concentration. A ratio of 10:1 was found to be preferable from the point of view of both handling, convenience and yield.

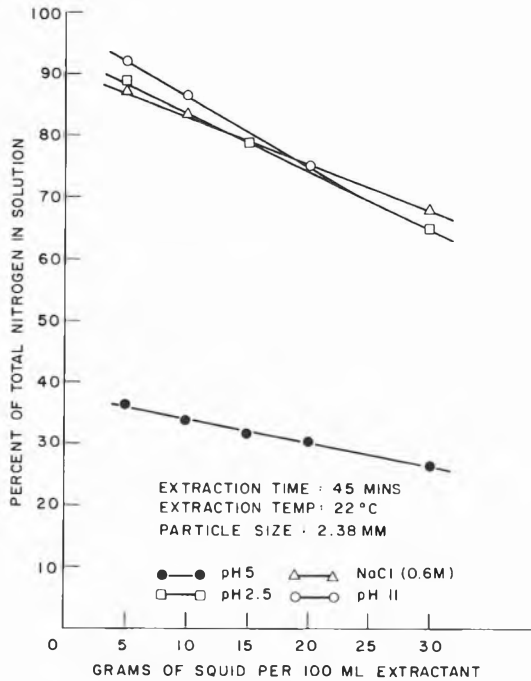


Fig. 4—Effect of the ratio of weight of squid to volume of extracting medium on the amount of protein extracted.

**Effect of extraction temperature on the amount of protein extracted**

The data (Fig. 5) indicate differences in the temperature extractability relationship of squid protein.

Extraction with 0.6M NaCl and in aqueous solutions at pH 2.5 and 11.0 showed that the largest amount of protein was in solution at 22°C. This was most marked with the 0.6M NaCl solution. At pH 5.0 slightly more protein was solubilized at the higher temperature.

A cooked odor and milky color were noticeable with extractions at higher temperatures (45°C and 60°C).

**Effect of the type and concentration of salt on the solubility of squid protein**

The results (Fig. 6) show that the greatest solubilization of protein occurred with sodium hexametaphosphate and sodium chloride, both at the 4% concentration level in the aqueous extractant.

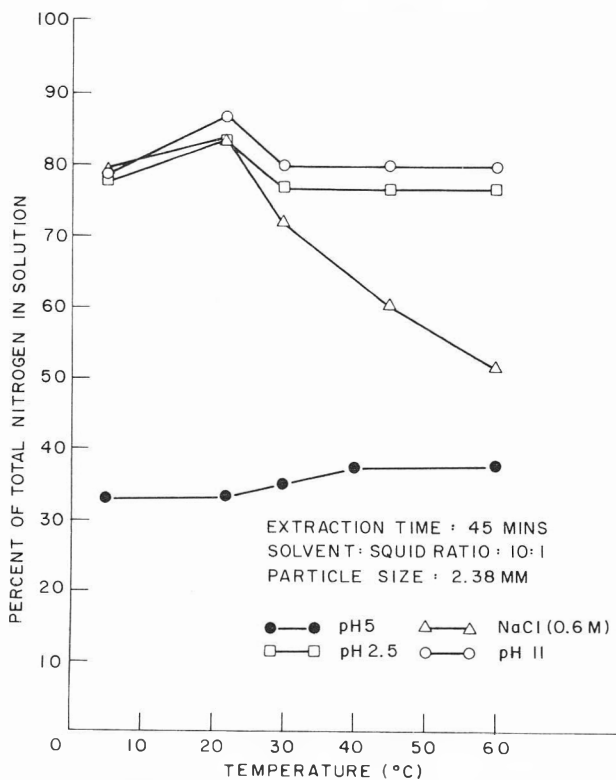


Fig. 5—Effect of extraction temperature on the amount of protein extracted.

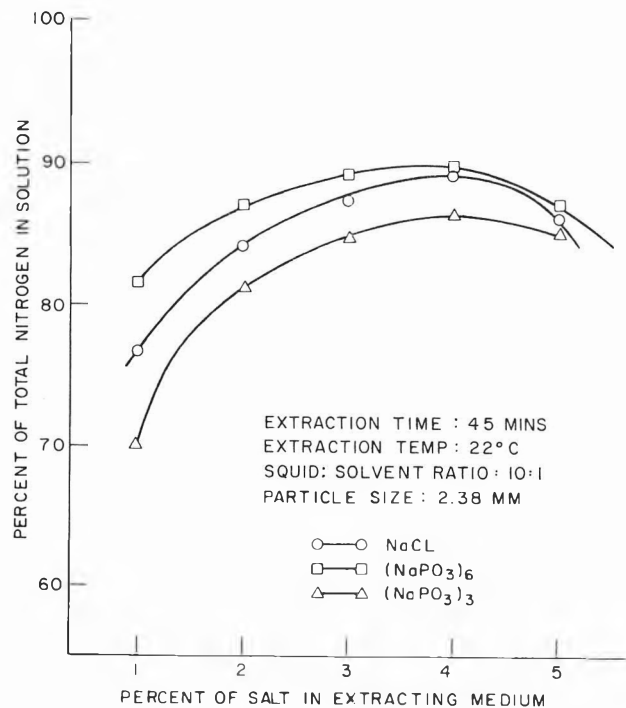


Fig. 6—Effect of type and concentration of salt on solubility of protein from squid.



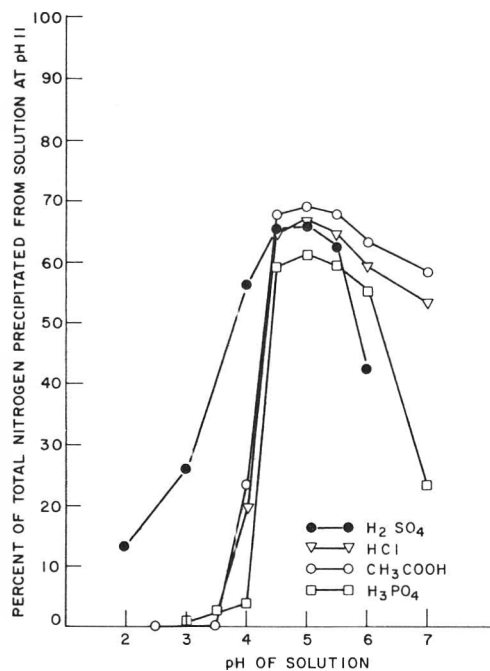


Fig. 7—Isoelectric precipitation of protein with selected acids.

The same trend was shown with sodium trimetaphosphate, but at lower extraction levels.

The good extraction yields obtained with condensed phosphate is in agreement with data reported by Spinelli and Koury (1970). However, sodium chloride seems to perform equally well as an extractant. For economic and safety reasons, sodium chloride is to be preferred.

#### Isoelectric precipitation of the protein

Figure 7 shows the amount of protein precipitated from an extract at pH 11 by acidification to various pH values. Approximately 2/3 of the extracted protein is precipitated at pH 4.5–5.5.

Maximum precipitation occurs at pH 5.0, which is also the point of minimum solubility (Fig. 3). Since approximately 1/3 of the extracted nitrogen remains in the whey after isoelectric precipitation at room temperature, recovery of this fraction may be worthwhile and will require different procedures.

It was found that the type of acid does not greatly influence precipitation yields.

#### Recovery of the isolates

Recovery of the isolates was carried

out on a pilot plant scale, using 50 liter extraction batches.

The isoelectric "curd" was spray dried (Anhydro Spray Drier, Copenhagen), at an inlet and outlet temperature of 220°C and 85°C, respectively.

The salt extract was desalinated using a pilot plant ultrafiltration unit (Abcor, Cambridge, Mass.) until the salt/protein ratio in the extract was reduced from the initial value of approximately 2.8 to a final value of approximately 0.2. The desalination extract was then spray dried as described above.

Process details of pilot plant runs as well as the properties of the products will be presented in a separate report.

#### DISCUSSION

OVER 80% of the squid protein is extractable in alkaline medium or salt solutions. Extraction yields in water at pH 7.0 were below 70%, in contradiction with values of 77–85% reported by Matsumoto (1958). An isolated protein can be obtained from extracts by isoelectric precipitation or by direct dehydration after salt removal. This study of the effects of various process parameters on

protein extraction yields illustrates the factors that bear importantly upon the extraction. From this information the following process parameters seem to be acceptable:

Particle size	2–3 mm
Extraction time	45 min
Extraction temp	22°C
Solvent-to-squid ratio	10:1 v/w
Extractant	4% NaCl or NaOH at pH 11
Recovery	Membrane desalination followed by spray drying or spray drying of washed isoelectric curd, precipitated at pH 5.

In future work, the effect of processing conditions on the functional and organoleptic properties of the isolates will be determined.

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## EFFECT OF SMOKING TEMPERATURES ON ACCEPTABILITY AND STORAGE STABILITY OF SMOKED SPANISH MACKEREL

### INTRODUCTION

SMOKING is one of the oldest methods for preserving fish. The main factors attributed to be responsible for the preservative effects of the process are the dehydration that occurs during smoking; the bactericidal properties (Hess, 1929; Gibbons et al., 1954); and the antioxidant properties (Banks, 1952) of the smoke. Salting is a preliminary treatment used either to provide a salty flavor or impart storage stability by decreasing the water activity. Smoke vapor constituents responsible for imparting biological stability to the product are phenolic compounds, acids and carbonyls (Porter et al., 1965; Foster and Simpson, 1961; Husini and Cooper, 1957; Shewan, 1949). Smoke flavor has been shown to be due primarily to volatile phenolic compounds in the vapor phase of the smoke (Bratzler et al., 1969).

Different types of smoked fish products are presently produced. The differences in process employed depend primarily upon the type of fish and regional preferences for a particular product. For most fish available in western Europe, the properties of the smoked products are well known and the appropriate time and temperature for smoking are well established. The time and temperature for smoking in a Torrey Kiln are given by Cutting (1951). Different schedules for different fish species are specified.

Recently, the Food and Drug Administration (FDA) published good manufacturing practices (GMP) for smoked fish which specified the minimum salt concentrations that must be present in the product smoked either cold or hot (FDA, 1970). This study was conducted to determine smoking procedures that would meet FDA's GMP for smoked fish and the effects of such processes on product quality. Spanish mackerel (*Scomberomorus maculatus*), a fish species found in abundant quantities in coastal water of the southeastern United States was used in the study since this species is presently not commercially smoked on a large scale, and the development of a suitable process would lead to more efficient utilization of this fishery resource.

### MATERIALS & METHODS

#### Smokehouse

The smokehouse used was a standard gas

fired one-cage smokehouse (Alkar, Lodi, Wis.) equipped with a smoke generator where smoke is passed over water to remove tar and solid particles (Model MGT, Meat Packers Equip. Co., Oakland, Calif.). Rate of air movement through the smokehouse was measured to be approximately 10 air changes per minute as calculated from the air velocity through the delivery duct and the volume of the empty smokehouse. Actual air velocities through the product were not precisely measured since air was introduced alternatively through one of two inlet vents and air flow was an eddy type mixed flow with cyclic highs and lows at any point.

#### Mackerel

Fresh mackerel were obtained from a wholesale fish dealer in Savannah, Ga., or collected from the shrimp boats by University of Georgia Marine Extension personnel in Brunswick, Ga. The fish were cleaned, filleted, packed in ice in insulated containers and transported by truck to Athens. The fish were received not more than 48 hr from the time they were harvested. They were either processed immediately upon receipt or quick frozen in  $-40^{\circ}\text{F}$  air blast and stored at  $0^{\circ}\text{F}$  until used. When frozen, the fish were thawed by immersion in running cold water for about 4 hr and immediately processed after thawing.

#### Brining

Brining was accomplished at  $75^{\circ}\text{F}$  by adding the fish to 23% NaCl solution at a ratio of 1 lb fish for every 0.7 lb of solution. After the desired contact time, the salt solution was drained and the fish were immediately rinsed by transferring about two layers deep into trays with false bottoms and sprayed approximately 5 sec with cold fresh water.

#### Equilibration

Two equilibration periods were employed in the process: one, immediately after brining, and the other between cold smoking and hot smoking. In both equilibration periods, the fish were hung on racks and introduced into a cold room maintained at  $38^{\circ}\text{F}$  and 70% relative humidity. Air circulation through the fish was kept at a minimum during the equilibration period and the only air movement was due to a blower which slowly circulated air through the refrigeration coils for cooling the room. The fish were not in the direct path of the air discharged from the refrigeration coils.

#### Cold smoking

Cold smoking was carried out by operating the smokehouse with the heating system turned off. Ambient air was heated slightly due to the smoke and the mechanical action of the blower. Therefore, the fresh air vent on the intake side of the duct for recirculating air was fully opened to supply sufficient fresh air to keep the temperature and humidity at a minimum. Air temperature averaged  $100^{\circ}\text{F}$  and relative humidity (RH) was 50–60%. RH was measured

using an electric hygrometer (Hydrodynamics, Inc., Silver Springs, Md.). Since the smokehouse was installed inside an air conditioned building, ambient conditions varied only slightly during the experiments.

#### Hot smoking

Hot smoking was carried out at  $160^{\circ}$ ,  $180^{\circ}$ ,  $200^{\circ}$  and  $210^{\circ}\text{F}$  dry bulb and 140, 159, 179 and  $159^{\circ}\text{F}$  wet bulb temperatures, respectively. This corresponds to a 60% RH except for the  $210^{\circ}\text{F}$  treatment which was at 29% RH.

#### Storage

After hot smoking the fish was immediately introduced into a  $38^{\circ}\text{F}$  room while still hanging on racks. They were allowed to cool (ca. 4 hr) and packaged in 1.5 mil thick polyethylene bags. The packaged fish were packed in fiberboard cartons, and stored at either  $38^{\circ}\text{F}$  or  $10^{\circ}\text{F}$ .

#### Panel evaluations of product quality

Subjective evaluations of product acceptability were carried out by panels composed of at least 10 students and staff from the Food Science Department. The nationalities and backgrounds of the panelists included Oriental, European, and Southeastern, Northeastern and Midwestern Americans. Statistical analysis of taste panel results showed no significant differences in responses among panelists.

For evaluations of color and appearance, the products were thawed if frozen, and presented to the panel, whole. For flavor evaluations, the products were thawed, cut into approximately 1/2 by 1 in. segments, placed in a dish covered with aluminum foil, heated in an oven at  $350^{\circ}\text{F}$  for 15 min, and allowed to cool to room temperature before presentation to the panel.

Panel members scored overall acceptance and appearance on a 7-point hedonic scale with 1 designating undesirable and 7 excellent. Salt, texture, smoke flavor and color were also evaluated on a 7-point scale with 1 being least, 4 just right and 7 excessive.

Statistical analyses were conducted on at least three judgments per panelist for each set of experimental conditions with each set repeated twice.

#### Microbiological analysis

Experiments on survival or proliferation of microorganisms on the product surface during processing were carried out by inoculating fillets with a 48-hr actively growing cell suspension of *Escherichia coli* just prior to cold smoking. 5 ml of the suspension was applied in small drops distributed uniformly throughout the product surface and spread out evenly using a glass rod. Two samples from each fillet were taken by using a flamed cork borer and the microbiological count was determined on TGE agar (Difco) using standard techniques. Microbiological counts on stored uninoculated smoked fish were determined using samples taken from the surface layer and the interior.

The fillets were sectioned and samples were taken using a flamed scalpel. Total aerobic counts of mesophilic and psychrophilic organisms per gram of sample were determined using standard techniques on nutrient agar (Difco). Mold counts were determined by plating on potato dextrose agar (Difco) and incubating at 21°C.

**Chemical analysis**

Oxidative rancidity was determined using the 2-thiobarbituric acid technique (TBA) reported by Yu and Sinnhuber (1957) and Sinnhuber and Yu (1958). Sodium chloride and moisture were determined using AOAC procedures (1970).

**RESULTS & DISCUSSION**

**Brining**

Figure 1 shows the water phase salt content [(NaCl × 100)/(NaCl + H<sub>2</sub>O)] defined by FDA (1970) and the corresponding taste panel scores for saltiness. The water phase salt content judged "just right" was 3.7%. FDA (1970) requires a minimum of 3.5% water phase salt in hot smoked fish and 5% in cold smoked fish. The ideal salt concentration is compatible with the requirement for a hot smoked product but is lower than what is required for a cold smoked product.

The use of 23% (w/w) NaCl solution for brining fishery products was recommended by Burgess and Cutting (1951) and Borgstrom (1965). The time of contact with 23% NaCl that is necessary to achieve the various salt concentrations in mackerel fillets which came from fish weighing 2-2.5 lb in the round is also shown in Figure 1. 4 min of contact were

necessary to obtain the ideal salt content. Various smoking schedules with hot smoking at 160°F and 60% RH (3 hr cold smoke + 1 hr hot smoke; 4 hr hot smoke; 3 hr cold smoke + 24 hr hold + 1 hr hot smoke; and 3 hr cold smoke + 48 hr hold + 1 hr hot smoke) following the 4 min brine dip, did not give significant differences in the water phase salt content of the smoked product.

Contact time in 23% brine found experimentally to be sufficient for various fish sizes were 2, 3, 4 and 5 min, respectively, for fillets from fish weighing 1-1.5 lb, 1.6-2 lb, 2.1-2.5 lb and 2.6-3 lb. The division of the weight classes here was arbitrary, but as a guide for determining brine dip times for various fish sizes, it has been shown to produce very satisfactory results. To be more precise, brining time should be based on the thickness of the fillets. Measurements of fillet thicknesses from Spanish mackerel as a function of fish weight in the round gave a regression equation (correlation coefficient = 0.887) of:

$$t = 0.18W + 0.45$$

where: t and W are fillet thickness and weight of fish respectively. The average

thickness of fillets from 2-2.5 lb fish was 0.86 in. The necessary brining time for any size fish can be calculated by:  $4 \times (t_{avg})^2 / (0.86)^2$ , since 4 min was required for 0.86 in. fillets (viz., Fig. 1) and solute uptake into the fillets is a function of the square of the thickness (DelValle and Nickerson, 1967). Where:  $t_{avg}$  is the average thickness of the fish.

**Effect of smoking temperature on acceptability**

Cold smoking, hot smoking, or combined hot and cold smoking (viz., Fig. 2) did not significantly affect saltiness, smoke flavor and color. However, significant differences were observed on texture and appearance, and these were sufficient to give significant differences in overall acceptance of the product.

The product smoked only at 160°F was significantly different in texture and overall acceptance compared to the other two processes. It had the softest texture and the least overall acceptance score. Between the cold smoked and the combined cold and hot smoked process, appearance was significantly different with the combination process scoring the highest in appearance.

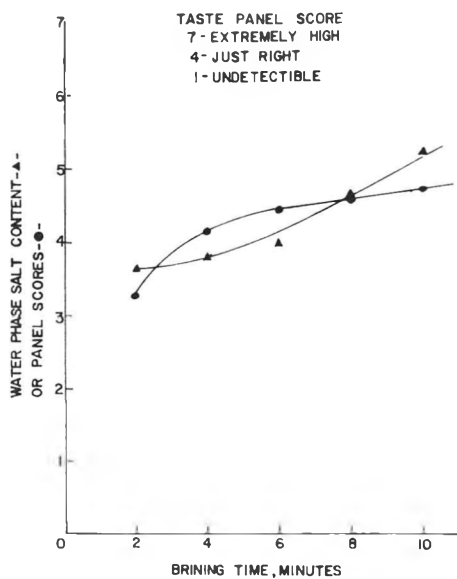


Fig. 1-Saltiness of smoked mackerel as affected by time of dip in 23% NaCl solution.

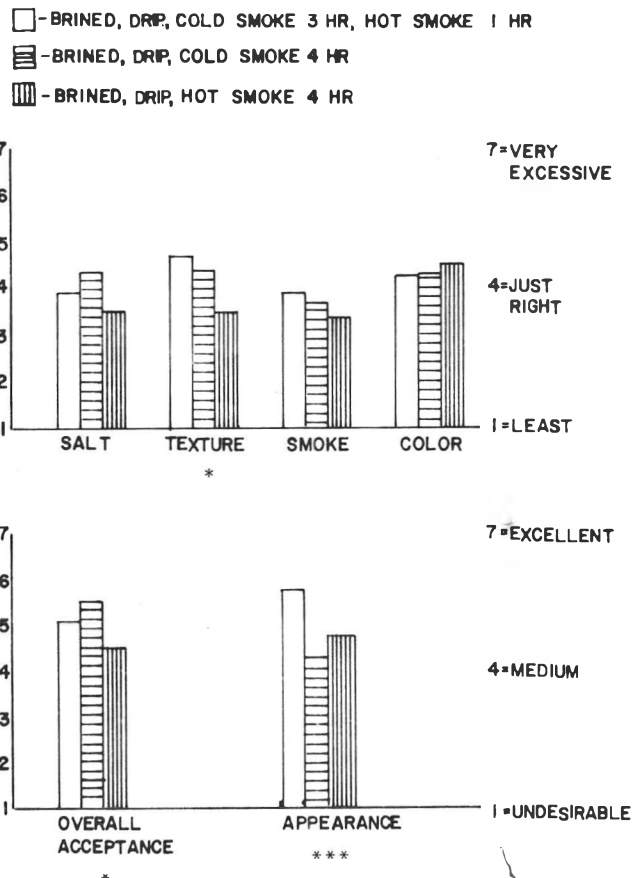


Fig. 2-Effect of smoking temperature on quality of smoked mackerel. (\* and \*\*\* denote, respectively, differences at  $\alpha = 0.1$  and 0.01.)

The softer texture of the hot smoked process was due to the high temperature of smoking at the time when the moisture content of the fish was high. The poor appearance score for the cold smoked product was due to the translucent hue of raw meat. In the combination process, the texture was comparable to the cold smoked process and the color was equivalent to that in the hot smoked product thus combining the best qualities of both.

Moisture contents of the smoked products ranged between 61 and 64% and there was no significant difference in the moisture contents of the products tested. Apparently the dehydration rate under the conditions used was controlled by mass transfer to such an extent that air temperature had no significant effect on extent of dehydration.

The recommended GMP for smoked fish (FDA, 1970) requires a minimum temperature throughout each fish of 180°F for 30 min when the water phase salt content is 3.5%. This apparently is based on thermal inactivation of *Clostridium botulinum* type E spores which has a "D" value of 1.7 min at 180°F and "Z" value of 16.5°F in phosphate buffer at pH 7. The substrate would have an effect on the "D" and "Z" values, but these were the highest values reported among three strains heated in saline, liver broth with liver particles, phosphate broth at pH 7, and tryptone-glucose-peptone broth (Schmidt, 1964).

The heating characteristics in a smokehouse of 0.85 in. thick mackerel fillets following a cold smoking process and holding at 38°F, are shown in Figure 3. All curves showed an initial rapid heating rate. Except for the 210°F process, the rate of temperature increase slowed down as the wet bulb temperature was reached. A semilogarithmic plot of dry bulb temperature minus fish temperature against time, showed a linear rise until the wet bulb temperature was approached, then a gradual leveling off of the curve occurred. This suggests that a faster rate of heating and a longer residence time at elevated internal temperatures could result if a higher humidity is used. However, results obtained in experiments we have conducted at higher humidities showed that the product texture was too soft because of insufficient dehydration, and the surface color was darker and duller than the product smoked at 60% RH at the same dry bulb temperature.

Equivalent heating times at 180°F for the transient heating curves in Figure 3 evaluated at the internal temperature for a "Z" value of 165°F are: 0.2, 4.6, 41.5 and 50.6 min respectively for the 160, 180, 200 and 210°F processes. These values were calculated using procedures similar to that used in evaluation of thermal processes in canned foods (Ball

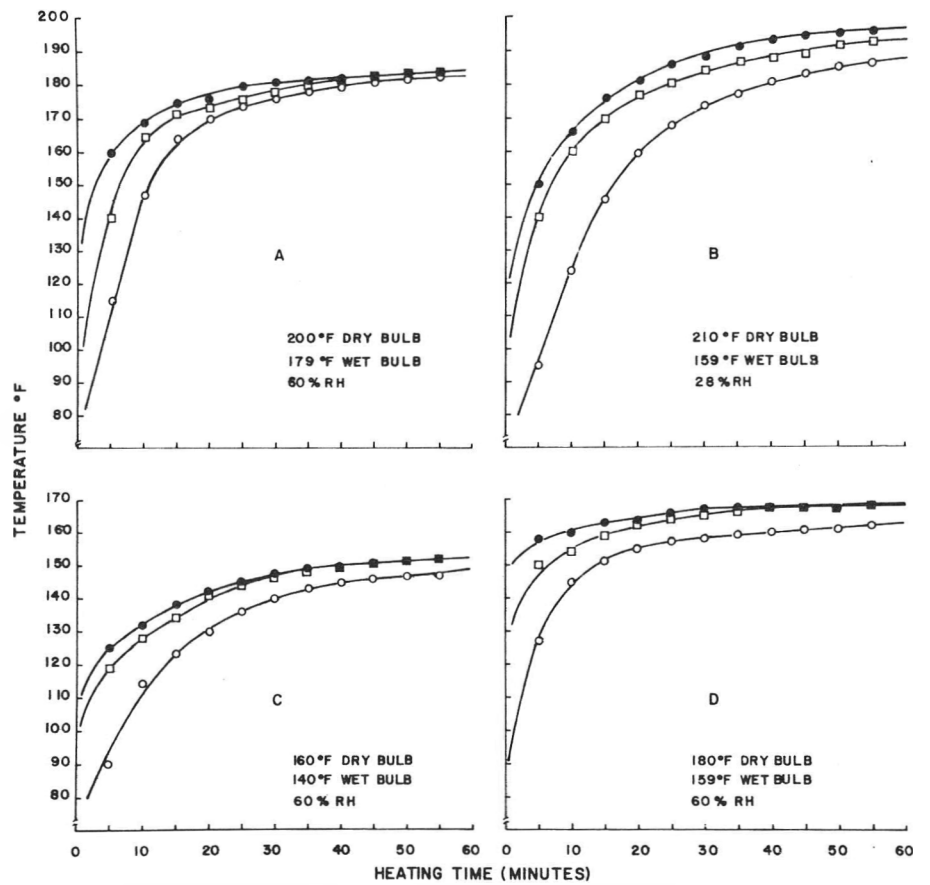


Fig. 3—Temperature change in fish when smoked at various smokehouse temperatures. (—○—○— internal; —□—□— underneath skin; —●—●— surface.)

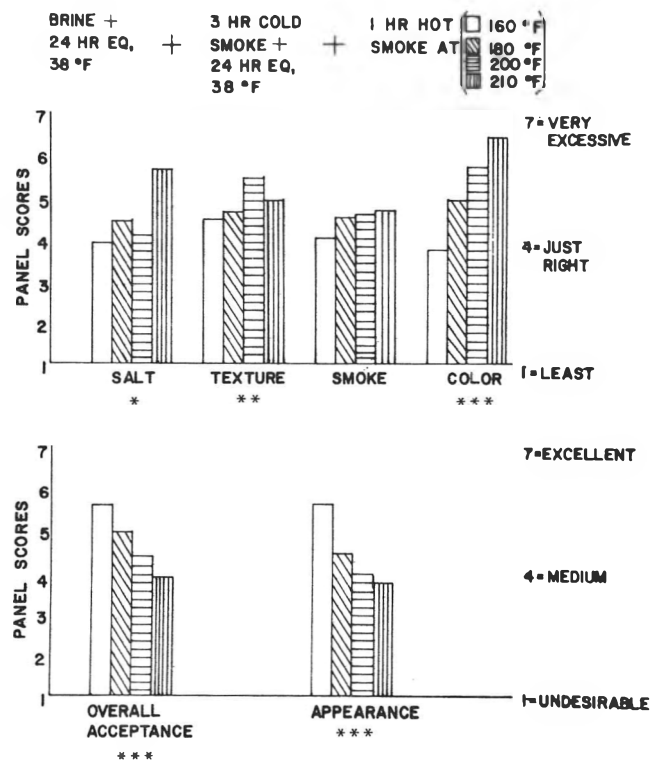


Fig. 4—Effect of smoking temperature on product quality in a combined cold and hot smoking process. (\*, \*\* and \*\*\* denote, respectively, differences between samples at  $\alpha = 0.1, 0.05$  and  $0.01$ .)

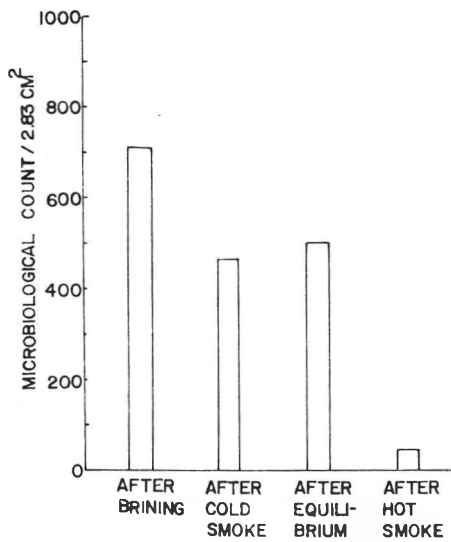


Fig. 5—Changes in microbiological count on surface of mackerel fillets during the smoking process.

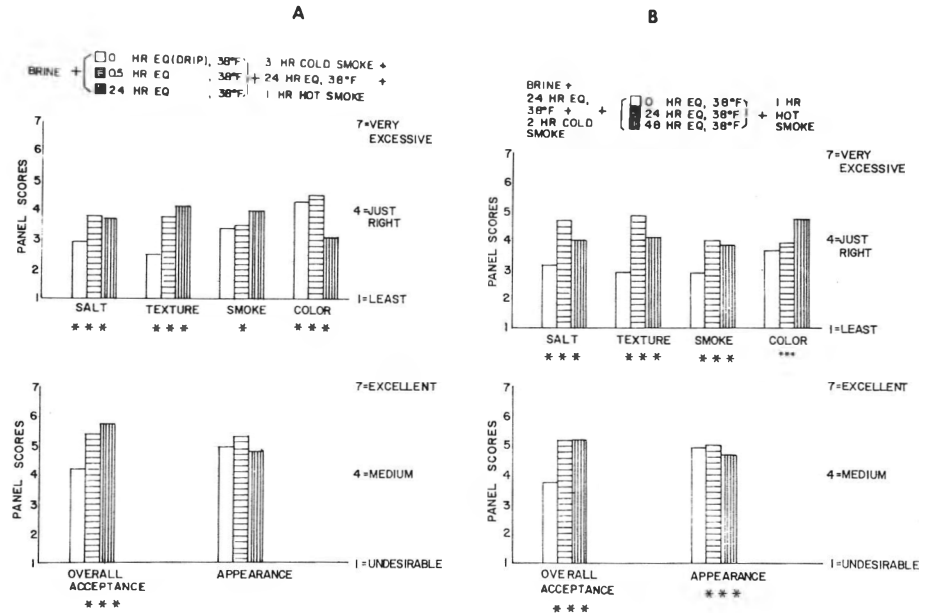


Fig. 6—Organoleptic quality of smoked mackerel when held for different time intervals after brining (A) and cold smoking (B). (\* and \*\*\*) denote, respectively, differences between samples at  $\alpha = 0.1$  and  $0.01$ .)

and Olson, 1957). A 12-D process for *Clostridium botulinum* type E is 30.4 min at 180°F (Schmidt, 1964). Thus, the 200 and 210°F processes meet the 12-D requirement but the 180 and 160°F processes are insufficient.

The effect of the higher heating temperature on product quality is shown in Figure 4. The product smoked at 160°F was significantly different and superior to all the other processes in all categories. The higher smoking temperatures resulted to darker color, a heightened smoke and salt flavor and a tougher texture. Overall acceptance scores were in an inverse relationship with smoking temperature.

**Microbiological changes during smoking**

Cold smoking at 100°F would enable microbiological proliferation if salting is insufficient and smoke deposition and dehydration rate is slow. Cold smoking at the low humidity and rapid air flow utilized in this study (Fig. 5) retarded microbiological activity by showing a slight decrease in surface counts. The action of salt, smoke and dehydration, however, was not sufficient to reduce surface counts significantly. Hot smoking at 160°F on the other hand, caused a very large reduction in count of vegetative microorganisms.

**Effect of length of equilibration period on quality**

The length of the holding period after brining (Fig. 6A) and between cold and

hot smoking (Fig. 6B) showed a definite effect on product acceptability. The quality of the product was much better when an equilibration period was used. A minimum of 0.5 hr equilibration after brining and 24 hr between hot and cold smoking is required for optimum results. Longer equilibration periods appear to

change product acceptability only very slightly over that obtained using the minimum.

**Storage stability**

Storage stability of the product at 38°F and 10°F was evaluated by taste panel (Fig. 7A and 7B); rancidity devel-

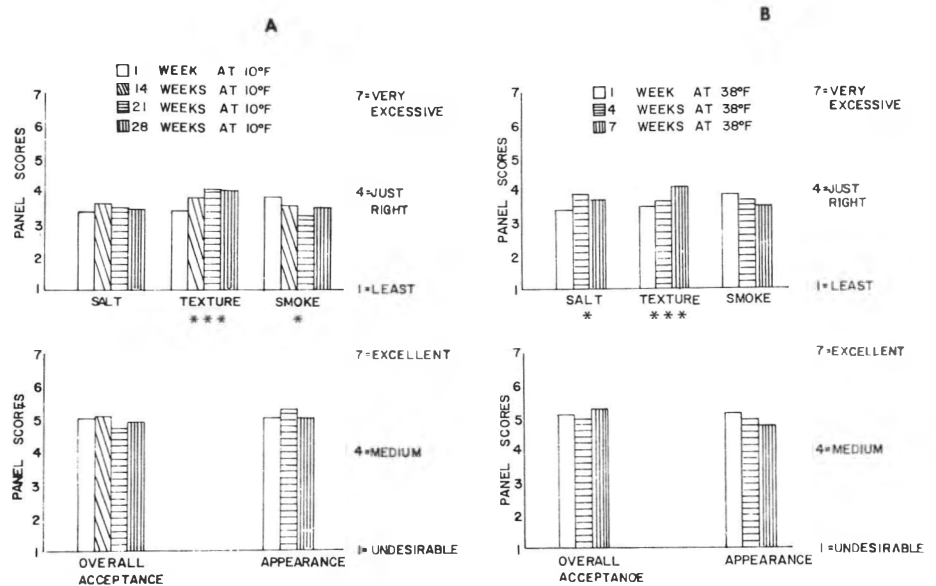


Fig. 7—Organoleptic quality of smoked mackerel when stored at 10°F (A) and 38°F (B). (\* and \*\*\*) denote, respectively, differences between samples at  $\alpha = 0.1$  and  $0.01$ .)

Table 1—Aerobic microbiological counts in smoked Spanish mackerel during storage at 38° F

Smoke time	Incubation temp (°C)	Surface				Interior			
		Days Storage				Days Storage			
		0	15	45	65	0	15	45	65
2 hr C.S.	32	0	5	9.0 X 10 <sup>3</sup>	8.5 X 10 <sup>6</sup>	40	10	80	7.8 X 10 <sup>6</sup>
	21 (mold)	0	0	9.0 X 10 <sup>2</sup>	1.0 X 10 <sup>3</sup>	—	—	—	—
1 hr H.S.	7	0	0	3.4 X 10 <sup>3</sup>	9.5 X 10 <sup>5</sup>	0	0	0	5.8 X 10 <sup>4</sup>
3 hr C.S.	32	0	25	4.6 X 10 <sup>2</sup>	1.6 X 10 <sup>6</sup>	0	10	1.1 X 10 <sup>2</sup>	1.5 X 10 <sup>6</sup>
	21 (mold)	0	0	< 10	2.9 X 10 <sup>2</sup>	—	—	—	—
1 hr H.S.	7	0	0	< 10	3.1 X 10 <sup>6</sup>	0	0	2.0 X 10 <sup>1</sup>	7.1 X 10 <sup>5</sup>
4 hr C.S.	32	0	1.0 X 10 <sup>2</sup>	1.8 X 10 <sup>3</sup>	9.1 X 10 <sup>5</sup>	0	40	10	1.0 X 10 <sup>6</sup>
	21 (mold)	0	0	1.5 X 10 <sup>2</sup>	4.1 X 10 <sup>2</sup>	—	—	—	—
1 hr H.S.	7	0	0	2.7 X 10 <sup>2</sup>	7.2 X 10 <sup>6</sup>	0	0	0	7.0 X 10 <sup>5</sup>
5 hr C.S.	32	0	70	7.4 X 10 <sup>2</sup>	1.3 X 10 <sup>4</sup>	25	30	2.5 X 10 <sup>2</sup>	1.4 X 10 <sup>5</sup>
	21 (mold)	0	0	< 10	2.5 X 10 <sup>2</sup>	—	—	—	—
1 hr H.S.	7	0	0	< 10	4.4 X 10 <sup>5</sup>	0	0	0	1.8 X 10 <sup>5</sup>

opment by TBA (Fig. 8A and 8B); and microbiological activity (Table 1).

Taste panel scores (products were prepared by dipping 4 min in 23% NaCl, 24 hr equilibration, 3 hr cold smoke, 24 hr equilibration and 1 hr hot smoke at 160°F) showed an insignificant change in overall acceptance after 7 and 28 wk at 38 and 10°F, respectively. A slight toughening of texture and a slight decrease in intensity of smoke flavor could be detected with prolonged storage.

TBA numbers of stored smoked Spanish mackerel showed a more rapid increase at 38°F storage compared to 10°F, but the increase after 10 wk at 38°F and 23 wk at 10°F was so small that rancidity development was not detected by the taste panel. No trend was observed on the difference in storage stability as affected by different cold smoking times in the process used.

Counts of aerobic microorganism on the surface layer of samples stored at 10°F were very low (< 10 per gram) and there was no significant increase in count with prolonged storage. Samples stored at 38°F (Table 1) showed increasing numbers with prolonged storage with a very steep exponential rise after 40 days in storage. The sample which was cold smoked the longest had the lowest rate of microbiological proliferation. Mold growth was apparent after 45 days at 38°F on some samples. This is shown by the increased numbers at 45 days storage at an incubation temperature of 21°C (70°F) in Table 1.

Although the heat treatment in the 160°F process is less than what is required to inactivate bacterial spores, the product is of excellent quality and had reasonable storage stability under refrigeration. The process, however, does not

meet FDA's GMP for smoked fish which requires a minimum of 30 min at 180°F fish temperature. Storage temperatures must be carefully controlled such that product temperature does not exceed 38°F. Since freezing does not alter product quality, frozen storage and distribution are recommended.

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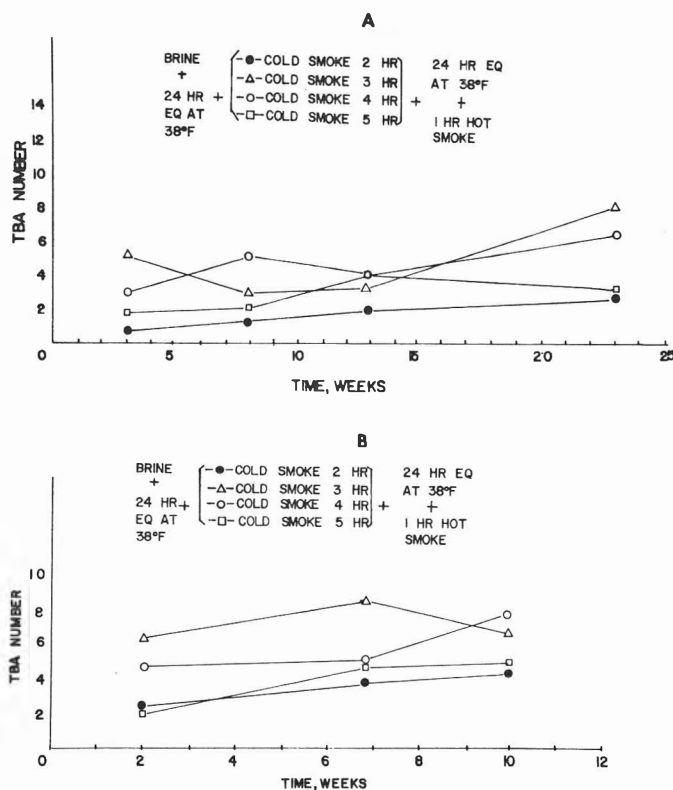


Fig. 8—Rancidity development in stored smoked mackerel as measured by the TBA number. A and B denote respectively storage at 10° F and 38° F.

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## EFFECT OF SMOKEHOUSE TEMPERATURE, HUMIDITY AND AIR VELOCITY ON RATE OF HEATING AND QUALITY OF FRANKFURTERS

### INTRODUCTION

MODERN SMOKEHOUSES and systems for cooking with hot air are equipped with instruments for programming of air temperature and for control of humidity and velocity, thus, offering potential for optimization of heat processing schedules. Optimization requires the application of fundamental heating principles in processing and the identification of their effect on product characteristics.

In general, high temperature and high humidity have been shown to have adverse effects on emulsion stability, texture and color development. These process conditions, however, resulted in decreased shrink, enhanced peelability, reduced cooking time and increased permeability of casings to smoke (Saffle et al., 1967; Simon et al., 1965; Tauber and Simon, 1963; Kramlich, 1972). Effects of air velocity have not been thoroughly explored except for some mention of it in the east European literature for which only abstracts are available, no results were presented, no references to text were supplied and attempts at personal communication brought no response (Babonov, 1966; Kulick et al., 1970).

With the advent of rapid and continuous processing the need for cure color development accelerators has intensified. Glucono delta lactone (GDL) has been suggested (Sair, 1965; Sair and Henry, 1967) and this compound is now used in the industry. Fox et al. (1967) found that addition of GDL had little effect on the color of conventionally processed frankfurters. Watanabe and Tape (1969) reported that color considered to be of commercial quality can be obtained in frankfurters containing 300 ppm GDL and heated by microwave energy in a process lasting 15 min from incorporation of curing ingredients. Sair and Henry (1967) recommended a minimum level of 2500 ppm and this apparent disagreement requires further affirmation. There is need for more data on color development acceleration by GDL in frankfurters processed rapidly in hot air systems.

The present study was conducted to determine the effects of temperature, humidity and air velocity on weight changes and temperature profiles in frankfurters; to establish the effects of these variables on quality; and to

compare the effect of GDL on color in rapid and conventional processes.

### EXPERIMENTAL

#### Experimental design

A four factor factorial experiment with two levels per factor was designed and executed with three replications. The factors and levels were: (1) No GDL or 2.5g GDL/kg of frankfurter formula; (2) dry bulb temperature constant at 93°C to a product internal temperature of 71°C or dry bulb temperature staged from 55°C at 5 or 6°C increments every 10 min to a product internal temperature of 71°C; (3) relative humidity (RH) of approximately 20 or 80%; and (4) air flow equivalent to 5 or 10 smokehouse air changes per minute.

The responses measured and analyzed were: (1) quantity of nitric oxide heme pigments; (2) color scores from panel evaluations; (3) texture scores from panel evaluations; (4) instrumental readings on product texture; (5) product shrink during smoking; and (6) temperature profiles and weight change during smoking.

#### Product preparation

The frankfurter formula (ca. 4 kg per batch) was:

lean cow meat	1230g
50/50 pk trim and jowels	1950g
crushed ice and water	794g
salt	95g
frankfurter seasoning	23g
sodium nitrite	0.51g
sodium nitrate	1.53g
ascorbic acid	1.75g
GDL (if used)	7.95g

Fresh meats were ground twice through a 4.8 mm (3/16 in.) plate, wrapped, stored at approximately -29°C, and allowed to thaw at 4°C for 24 hr before use. The batter was chopped in a 4 kg capacity Hobart silent cutter (model 31510) modified to allow the use of four rotating blades. The chopper bowl was pre-chilled with ice then the lean meat, ice water, nitrite and seasonings were chopped to a temperature of 4°C. The fat meat and the dissolved mixture of ascorbic acid and GDL (if used) was poured evenly into the chopping bowl with continued chopping until the mixture reached 10°C. Chopping was stopped, the batter passed through a colloid mill (Kolloid Technik), stuffed into 20-21 mm cellulosic casings (Tee-Pak), and linked in 12 cm lengths with a hand operated sausage linker (Griffith Laboratories). Two thermocouples positioned by means of plastic spacers were installed in one link of each batch to measure surface and center temperature. The thermocouples were positioned at a point 5 cm from one end of the link. The linked batches were weighed and hung on smokesticks.

Each batch was heat processed within 30 min after chopping following recommended procedures for GDL usage (Sair and Henry, 1967). The smokehouse experiments were carried out in random order. Batches with or without GDL and assigned identical heat treatments were heat processed at the same time. Heat processing was carried out in a pre-heated one-cage, air-conditioned smokehouse (Alkar). Internal and surface product temperatures during a process were recorded continuously on a process monitoring system (Morrow Systems). Weight change was continuously recorded by suspending one smoke stick with product from a wire hanging from the arm of a weight measuring device situated on the smokehouse roof. The device was a lever, free to swivel up and down on bearinged restraints at one end, with the load hung on the opposite end of the restraint. A compression ring taken from a shear press [Food Technology Corp. (FTC), Model T61A, 100-lb range] acted as the fulcrum. The force on the fulcrum was detected and amplified by the FTC Model TR1 amplifier system and was recorded on a strip chart recorder. The device was calibrated by hanging known weights at the same point the products were hung. All heat processes were followed by 5 min of mild steaming in still air, then given a 5 min cold shower. Each batch was weighed and placed under refrigeration at high RH at 3°C overnight. On the following morning, casings were peeled off by hand and batches were sampled for evaluation.

#### Product evaluation

**Chemical analysis of color.** Nitric oxide heme pigments were determined using a modification of the procedure of Hornsey (1956). 10g of frankfurter samples were extracted with 50 ml of 90% acetone-water solution, the extracts diluted to 100 ml and the O.D. read at 540 nm. Pigment content was computed by the formula: mg pigment/g tissue = O.D. × 15.04.

**Color panel.** An 8 to 13 member panel evaluated color using a 9-point hedonic scale with 9 being the most acceptable and 1 being colorless. Samples from the treatments were displayed together under uniform light from blue photographic light bulbs.

**Texture panel.** A 10 to 13 member panel evaluated color using a 9-point hedonic scale with 9 being most acceptable and 1 unacceptable. A panelist judged a random selection of 8 of the 16 samples at a morning session and the remaining 8 in the afternoon. Samples were prepared for the panel by bringing water to a boil, removing heat and immersing the frankfurters for 7 min.

**Instrument texture.** An incisor-shaped punch of the type introduced by Simon et al. (1965) was mounted on an Instron Universal Testing Instrument, Model TM. A 10-cm-long stainless steel V-shaped trough was constructed from a 1-1/4 × 1-1/4 × 1/4 in. angle and used



as a sample holder. The instrument was adjusted to give a chart and punch speed of 25.4 cm per min. The sample was oriented so that the punch would pass through the geometric center. The two values taken from the recording of the penetration of force that were considered to be indicative of frankfurter texture was: (1) the first peak height interpreted as lb of force required to break the skin and (2) the second peak height as the force required to penetrate the interior of the frankfurter.

**Shrinkage.** Shrinkage was measured immediately after the heat process and expressed as weight loss in percent of initial weight.

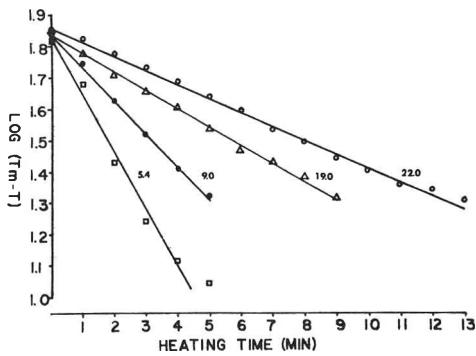


Fig. 1—Semilogarithmic plot of the difference between the smokehouse temperature ( $T_m$ ) and the frankfurter internal temperature ( $T$ ) for constant temperature ( $93^\circ\text{C}$ ) processes. The number associated with each curve is the slope index ( $f$ ). ( $\square$ , high humidity, low airflow;  $\bullet$ , high humidity, high airflow;  $\triangle$ , low humidity, low airflow;  $\circ$ , low humidity, high airflow.)

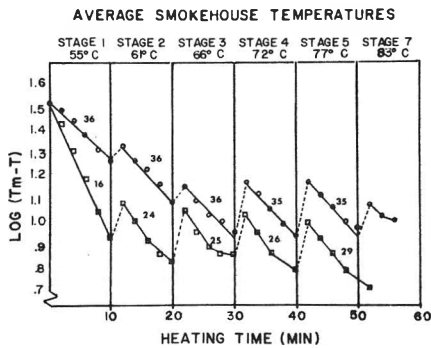


Fig. 2—Semilogarithmic plot of the difference between the smokehouse temperature ( $T_m$ ) and the frankfurter internal temperature ( $T$ ) for staged temperature processes. The number associated with each stage of each curve is the slope index ( $f$ ). ( $\square$ , low humidity;  $\square$ , high humidity.)

RESULTS & DISCUSSION

Effect of relative humidity and air flow on heating rate and weight loss

Increase in internal temperature of comminuted meat products contained in a water permeable casing and exposed to flowing air (Fig. 1), follows a straight line in a semilogarithmic plot against time. The type of curves obtained is similar to that for the internal temperature of cans processed in steam (Ball and Olson, 1957), except for the absence of a lag. The absence of a lag could be due to the very small distance from the center of the test piece to the surface. The slope indices  $f$  in Figure 1 are the times required for the curve to traverse 1 log cycle (following the notation of Ball and Olson, 1967) and is inversely proportional to the heating rate. Rate of heating was faster at 80% compared to 20% RH and for the same humidity, heating was faster at

slower air flow rates. This effect of humidity can be seen again in a staged process (Fig. 2). Slope indices  $f$  stayed about the same with each heating stage for the low humidity process whereas a markedly faster rate of heating is evident at the start of a high humidity process.

Figure 3 shows time vs. temperature curves for the staged process shown in Figure 2, together with the weight change during the process. Because of the lag involved from the time the controls were set to the time air temperature settled to a steady state value, the actual air temperature did not stay constant at a single temperature for each stage. The values for  $T_m$  used in plotting Figure 2 were time weighted average temperatures for each stage.

Rate of evaporation was markedly faster and rate of heating was slower in a product subjected to a low humidity process compared to that in a high humidity process. This indicates that water transfer

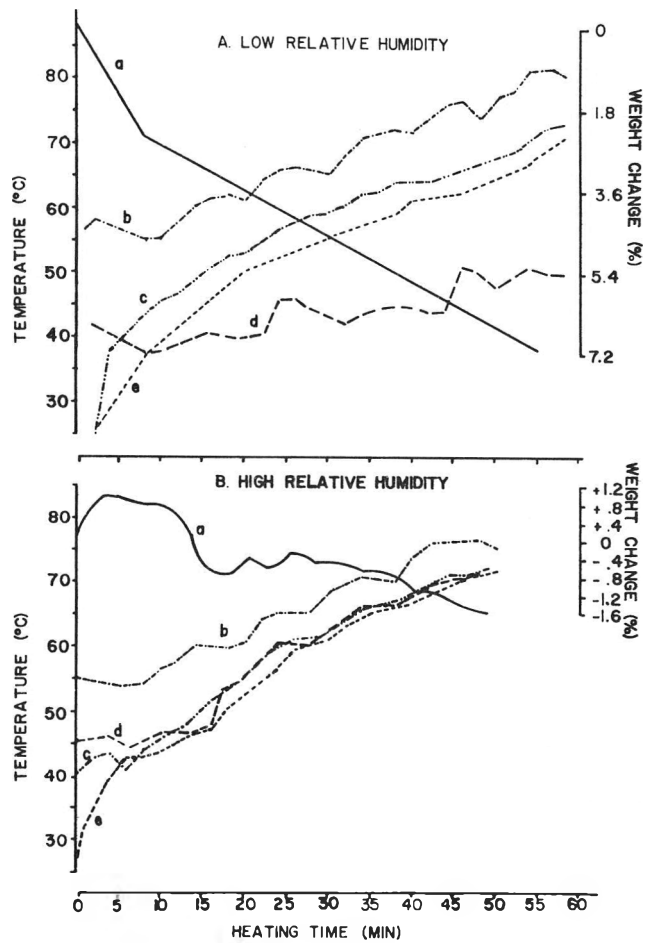


Fig. 3—Time vs. temperature plots for high and low humidity staged temperature processes with superimposed weight change record. (a, percent change in frankfurter weight; b, smokehouse dry bulb temperature; c, frankfurter surface temperature; d, smokehouse wet bulb temperature; e, frankfurter internal temperature.)

Table 1—Effect of smokehouse conditions and glucono delta lactone on frankfurter quality<sup>a</sup>

Measured responses	Factor							
	GDL		Air temperature		Relative humidity		Air flow (Changes/min)	
	None	2.47 g/Kg	93°C	Staged	20%	80%	10	5
Shrink %	6.98	7.37	6.30	8.06	8.18	6.18	7.72	6.64
Color score	5.2	5.1	4.7	5.6	5.5	4.8	5.3	5.0
Pigment (Mg/g tissue)	1.56	1.62	1.56	1.62	1.64	1.55	1.62	1.56
Texture score	6.43	6.48	6.26	6.66	6.57	6.34	6.58	6.33
Instrument surface texture (lb)	0.705	0.702	0.636	0.77	0.74	0.66	0.716	0.69
Instrument Body texture (lb)	1.24	1.29	1.21	1.33	1.31	1.23	1.30	1.24

<sup>a</sup> These tests of significance compare only the two treatment level means under a single factor. Means underscored by the same line are not significantly different. Means underscored separately by single lines are significantly different ( $P < 0.05$ ). Means underscored separately by double lines are significantly different ( $P < 0.01$ ).

and the accompanying latent heat plays a major role in the rate of heating of meat products contained in a water permeable casing and exposed to flowing hot air. Where a weight increase occurred (first 10 min, Fig. 3B) due to condensation on a cold surface from the hot humid air, rate of internal heating was significantly faster for this stage compared to the others. The faster weight loss in the first 7 min of the low humidity process (Fig. 3A) is not reflected by a change in heating rate but this could be due to the opposing effects of a rapid initial heat transfer to the cold frankfurter due to a high temperature driving force and the cooling effect of evaporation.

Saffle et al. (1967) explained the importance of RH in heat processing of frankfurter on the basis of increased BTU per pound of air at higher humidities. The results in the present study show that the difference in heating rates observed between a high RH and a low RH process is due to the accompanying latent heat of water transferred to or from a product during processing. A low RH process showed more moisture loss and a slower rate of heating. At constant temperature processes shown in Figure 1, The higher the air flow, the slower the rate of heating for the same RH. The relationship between product temperature, weight loss and smokehouse conditions are therefore analogous to what occurs during air dehydration as discussed by Van Arsdel (1963). In the case of frankfurters, protein coagulation, skin formation and other changes that alter the water immobilizing properties of the material apparently limit evaporation at the very

low RH since the surface temperature was higher than the wet bulb temperature of the air. However, at high RH, rate of evaporation was sufficiently slow to allow a balance between heat transfer and evaporative cooling. Thus, surface temperature followed very closely the wet bulb temperature of the air (Fig. 3).

#### Effects on product quality

The effects of velocity, humidity, temperature and GDL on color, texture and shrink are summarized in Table 1. All products except those processed with a combination of high humidity and high temperature would be commercially acceptable. Products processed at high RH and high temperature were unacceptable because of fat separation and pale color. In general, low humidity, a staged processing temperature, and high air flow resulted in greater color development, firmer and more acceptable texture and increased shrink. It appears that the color and texture effects may be caused directly by shrink with a heightened color resulting from increased concentration of solids.

GDL showed no significant effect on development of final color either in rapid or staged processes. When samples were withdrawn at 5 min intervals in a staged process and evaluated visually, those containing GDL reached maximum color development when internal temperature was only 57°C (25 min heating) and no further increase occurred with prolonged heating. Those samples without GDL did not reach maximum color development until the internal temperature reached 70°C (45 min heating). There were no

differences in the final color between samples with and without GDL. Fox et al. (1967) observed more intense color in the presence of GDL at 57°C finish temperature whereas no differences were found at 68°C finish temperature. Thus, if the processes are such that the same final internal temperature is attained and they last for a sufficiently long duration, color differences would not be noticeable between samples with and without GDL. In the 93°C process at 80% RH and slow air flow which had the shortest duration (5 min), the time and temperatures attained were not sufficient to permit full color development even in samples containing GDL. No significant effect of GDL on pigment concentration or panel color score was observed.

Surface texture and body texture measured objectively gave parallel results with taste panel scores for texture. The firmer samples received higher texture scores from the panel.

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## ACCELERATED PORK PROCESSING: A QUANTITATIVE STUDY OF BACTERIAL FLORA OF CURED AND SMOKED HAMS

### INTRODUCTION

THE PROCESSING of ham to a finished product prior to initial chilling has introduced a new concept to the meat industry. Several investigations have shown the desirability of accelerated, hot processing techniques in ham curing operations in the laboratory (Barbe et al., 1966; Mandigo and Henrickson, 1966). Since these techniques eliminate conventional chilling, the bacteriological status of the pork product has been questioned. The objective of this experiment was to quantitatively compare the bacterial flora common to conventional and accelerated processed pork products.

Experiments prior to this study were conducted in the laboratory. This study was designed not only to find differences between the rapid and conventional processing treatments, but also relate these treatments to a commercial processing schedule. Of main interest are the numbers and type of bacteria, and the influence of the rapid processing technique on finished pork products.

### MATERIALS & METHODS

225 BUTCHER HOGS with unknown breeding, feeding and management were selected at random from a local slaughter plant. Accelerated treatment or hot side refers to the randomly selected side (half) being fabricated prior to normal chilling, whereas conventional treatment or randomly selected cold side included a 24 hr initial chilling period at 1.7°C.

Following the product flow diagram illustrated in Fig. 1, all hams were stitch-pumped with a multiple needle process with commercial brines, using the industry methodology of the cooperating processing plant. The hams were skinned, outside fat removed, then boned and pumped. The hams were stuffed into individual fibrous casings, placed into stockettes, pressed in steel molds and moved into the smokehouse. After a 1 hr initial drying period, smoking was commenced and continued for 8 hr including cooking until the hams reached an internal temperature of 69.5°C. The smoked and fully cooked hams were removed from the smokehouse and cooled rapidly in the chilling chamber. The temperature of the chill chamber varied from -20°C to -25°C. Cooling time was adjusted so that the internal temperature reached approximately 10°C. The hams were

then moved to the cooler where they were maintained at the conventional meat holding temperature (1.7°C).

All bacteriological survey core samples were aseptically removed by a mechanical coring device autoclave sterilized and powered by a portable ¼ in. electric drill. The surface where the sample was to be taken was seared with a heat sealing iron. The sample was removed from the semimembranosus muscle immediately below the symphysis pubis and perpendicular to the muscle surface. All cured samples were cored horizontal to the flat face of the molded boneless ham at the midpoint of the longest side. All samples were placed immediately under refrigeration until preparation for bacteriological plating.

Total counts were determined by standard bacteriological methods. 11g of the inner portion of the cored sample was placed into a sterile glass blender jar containing 99 ml of sterile phosphate buffer (APHA, 1966). Samples were blended for 2 min at approximately 10,000 rpm. Prior to pipetting sample aliquots for plating, each dilution blank containing the homogenate and glass beads was shaken vigorously. Quadruplicate platings were made at serial dilutions of 10<sup>-2</sup> to 10<sup>-4</sup> using standard method agar (tryptone glucose yeast agar, TGYA). Duplicate sets of standard method

plates were incubated at 37°C with the remaining set being incubated at 15°C. After 3 days at 37°C and 5 days at 15°C, appropriate plates were counted and the number of aerobic bacteria per gram of original sample computed. Anaerobic bacteria (anaerobes and facultative anaerobes) were quantitated after incubation in an anaerobic incubator at 37°C (90% N, 10% CO<sub>2</sub>).

Anaerobic sporeformers were quantitated using the most probable number technique (MPN) for estimating total bacterial numbers. The procedures followed those of Steinkraus and Ayres, (1964) and Barbe et al. (1966). Enumeration followed pasteurization at 60°C for 55 min (Barbe et al., 1966) of the original homogenate from which the total and coliform counts had been plated. After pasteurization MPN counts using thioglycollate medium (with indicator) modified with a sulfide detecting agent (0.2% ferrous ammonium sulfate) were seeded in 5-tube replicates using dilutions corresponding to the volumes of 10, 1.0 and 0.1 ml of the original 1:10 meat homogenate. The tubes were cooled rapidly prior to seeding to expel residual oxygen. The tubes were incubated 5 days at 37°C. Only those tubes having a black precipitate (FeS) and producing a sour, putrefactive odor were recorded as positive (Barbe et al., 1966). The most probable number

Table 1—Aerobic bacteria, anaerobic bacteria and anaerobic sporeformer bacteria isolated from ham

Treatment	Aerobes/100g									
	< 10		10 to 50		50 to 250		250 to 500		> 500	
	n	%	n	%	n	%	n	%	n	%
Accelerated-fresh	35	73	13	27	0	0	0	0	0	0
Conventional-fresh	32	67	15	31	1	2	0	0	0	0
Accelerated-cured	12	25	11	23	9	19	16	33	0	0
Conventional-cured	16	33	10	21	8	18	14	29	0	0
Treatment	Anaerobes/100g									
	< 10		10 to 50		50 to 250		250 to 500		> 500	
	n	%	n	%	n	%	n	%	n	%
Accelerated-fresh	44	92	4	8	0	0	0	0	0	0
Conventional-fresh	46	96	0	0	1	2	1	2	0	0
Accelerated-cured	26	54	11	23	9	19	2	4	0	0
Conventional-cured	20	42	10	31	8	17	6	12	4	8
Treatment	Anaerobic sporeformers/100g									
	< 1		1 to 5		5 to 25		25 to 100		> 100	
	n	%	n	%	n	%	n	%	n	%
Accelerated-fresh	44	92	4	8	0	0	0	0	0	0
Conventional-fresh	44	92	2	4	2	4	0	0	0	0
Accelerated-cured	25	52	8	17	11	23	4	8	0	0
Conventional-cured	27	56	11	23	7	15	2	4	1	2

<sup>1</sup> Present address: Oscar Meyer Inc., Madison, Wis.

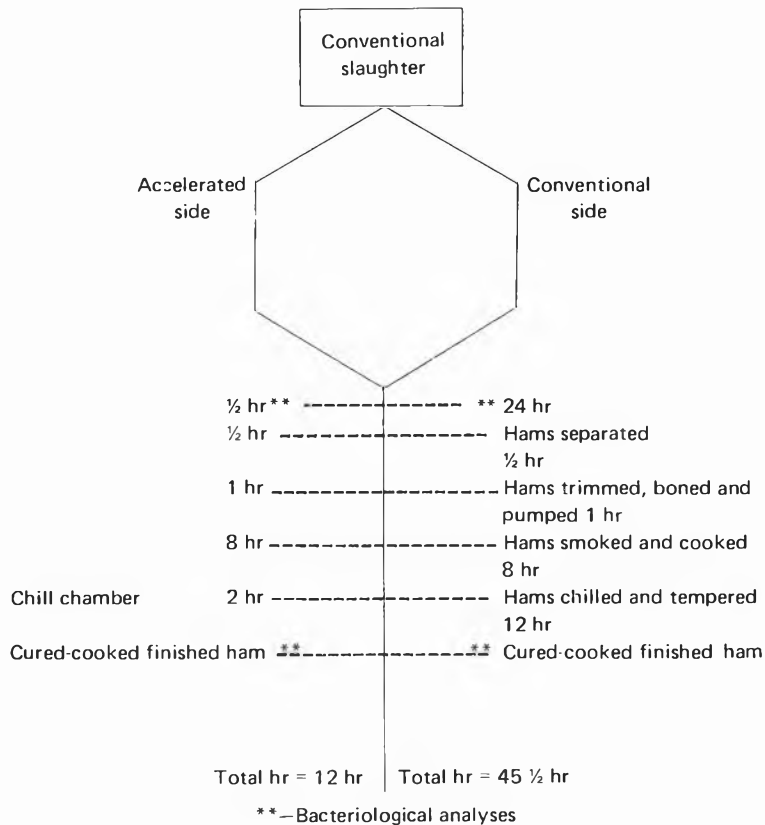


Fig. 1—Process flow diagram

of sporeforming anaerobes was estimated by referring to MPN tables (Halvorson and Ziegler, 1933).

## RESULTS & DISCUSSION

QUANTITATION of bacterial numbers are based on 48 pairs of hams. Table 1 shows that 73% of the accelerated hams had no colonies that could be detected, or contained extremely low numbers (< 10 per plate), as compared to the conventional processed hams where 67% of the uncured samples contained no countable growth. 25% of the cured accelerated hams and 33% of the conventional hams had little or no growth. Enumeration of psychrophiles resulted in only two conventionally processed uncured ham samples with viable growth.

The data are not normally distributed and are generally skewed toward zero. This necessitates use of nonparametric statistics, thus, the Wilcoxon matched-pairs signed ranks test was employed (Siegel, 1959). This experiment studies the difference between accelerated and conventional processing techniques as well as differences between uncured and cured hams. Three orthogonal comparisons were made: (1) accelerated vs. conventional within uncured fresh ham; (2) accelerated vs. conventional processing

within cured-cooked ham; and (3) uncured fresh ham vs. cured-cooked ham. Comparisons were made between accelerated and conventional processing techniques in fresh uncured ham to determine any significant difference between aerobic bacteria and anaerobic sporeformers. Results reveal no significant difference ( $P < 0.01$ ) between the two processing techniques. Another comparison was made between accelerated and conventional processing techniques in cured-cooked ham to determine any differences between aerobic bacteria, anaerobic bacteria and anaerobic sporeformers. Results reveal no significant difference ( $P < 0.01$ ) between the two processing techniques. Results did reveal a significantly higher count ( $P < 0.05$ ) in anaerobic bacteria in the conventional processed cured hams when compared to the accelerated technique. A third comparison was made between the uncured and cured ham. Cured hams were significantly higher ( $P < 0.01$ ) in microbial counts of aerobic and anaerobic bacteria and anaerobic sporeformers.

The flesh of freshly slaughtered healthy meat animals is generally considered to be nearly sterile, and a lower incidence of bacteria was found in the uncured ham in both treatments when compared to the

cured ham. The presence of the aerobic and facultative anaerobic organisms in the fresh (uncured), hot processed ham could be attributed to postmortem depletion of leucocytes and antibodies when muscle pH has not reached an inhibitory concentration (Frazier, 1958).

The low incidence of anaerobic spores estimated by the most probable numbers (MPN) technique are shown in Table 1. 92% of the samples in the two uncured treatments did not contain anaerobic spores capable of producing a black precipitate. Over 50% of the cured hams in both accelerated and conventional treatments revealed anaerobic spores. The presence of anaerobic spores in the tissue of only eight different uncured ham tissues out of the 96 surveyed is similar to the findings of Steinkraus and Ayres (1964) and Barbe et al. (1966). According to Strong et al. (1963) and Hall and Angelotti (1965) a level of less than 10 anaerobic spores per gram is most likely to be encountered as packing house contaminants. Accelerated processing could be as equally effective as normal procedures in reducing "in-process" contamination. A number of workers have shown that spores from spore forming bacilli can germinate at sub-minimal growth temperatures (20°C) (Mundt et al., 1954; Curran and Pallansch, 1963). The exposure of anaerobic spores to a rapid prechill (-20 to -25°C) may eliminate the germination of spores altogether.

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## EFFECT OF NITRITE AND STORAGE TEMPERATURE ON THE ORGANOLEPTIC QUALITY AND TOXINOGENESIS BY *Clostridium botulinum* IN VACUUM-PACKAGED SIDE BACON

### INTRODUCTION

CURRENT CONCERN over the use of nitrite as a food additive arises from the discovery that nitrites and amines can react to form nitrosamines (Magee and Barnes, 1967; Druckrey et al., 1967; Möhler and Mayrhofer, 1968). Nitrite is used as an additive in cured meats for color fixation (Fiddler et al., 1972) and development of characteristic flavors (Cho and Bratzler, 1970). It is also known that nitrite can be an important factor in the inhibition of *Clostridium botulinum* in certain cured meat products (Steinke and Foster, 1951; Silliker et al., 1958; Riemann, 1963; Koelensmid-Blanche and van Rhee, 1968). Evidence for this effect, however, was questioned by some research workers (Roberts, 1971; Tjoberg and Kvaale, 1972). It appears that any general assessment about the ability of nitrite in preventing formation of botulinum toxin may not be valid, unless the food product is specified. Since cured products differ in terms of processing, chemical composition and microbial ecology, the so-called "biological uniqueness" (Greenberg, 1972) of each product has to be considered.

The main objective of this study was to examine the role of nitrite in the inhibition of *C. botulinum* in vacuum-packaged side bacon.

### MATERIALS & METHODS

#### Experimental design

In a two-part experiment, bacon was prepared at five levels of nitrite (0–200 ppm) and inoculated with *Clostridium botulinum* spores at levels of  $10^2$  and  $10^4$  per g. In the first part of the experiment (A), 304 sliced bacon packs were inoculated and stored for 32 days at 20°C and 30°C. In the second part (B) a further 320 sliced bacon packs were inoculated and stored at 30°C for 32 days. At intervals during storage both sets of bacon packs were examined for appearance, odor and toxin. The relationship between nitrite and toxin formation was analyzed by the analysis of variance procedure.

<sup>1</sup> Microbiology Div., Food Research Labs, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, K1A 0L2

<sup>2</sup> R & D Labs, Canada Packers Limited, Toronto 9, Ontario

<sup>3</sup> Dept. of Agriculture, Food Research Institute, Canada Dept. of Agriculture, Experimental Farm, Ottawa, Ontario

#### Bacon preparation

A hand-pumping machine was used to inject trimmed fresh pork bellies. The pickle retained was about 9.0% of the fresh weight. The formulation in pounds per 100 gallons of the pickle was: salt 176; sugar 75; and sodium nitrite, 0, 0.69, 1.38, 2.07 or 2.76. These amounts of nitrite correspond to 0, 50, 100, 150 and 200 ppm. After pickling, the bellies were smoked for 8–10 hr to an internal temperature of 54°C and then chilled for 48 hr at –3°C. The bellies were then pressed, sliced and vacuum-packaged in 2.75 ml. nylon polyethylene laminate film (Cryovac 872 AX). Following overnight storage at 3°C the 8 oz packs were analyzed for salt, nitrite and moisture content.

#### Chemical analysis

Four packs of bacon per initial nitrite concentration were thoroughly ground. For the determination of the salt content, 5 ml of cold water and 25 ml of boiling water were added to 2g of the ground bacon. After stirring, 50 ml of 0.2M acetate buffer (pH 4.5) and 50 ml of acetone were added. The salt concentration was determined with a Fisher Titralyer (Fisher Scientific Co. Ltd.). Nitrite and moisture contents were determined by the official method of the AOAC (1970).

#### Inoculation of bacon

Spores of *Clostridium botulinum* 62A, TA, 54B and 13983B were prepared as described by Johnston et al. (1969). Bacon packs were inoculated with heat-activated (80°C for 15 min) spore suspensions in distilled water to a concentration of  $10^2$  or  $10^4$  spores/g. The spores were distributed between the bacon slices by the method of Pivnick and Bird (1965). Inoculated samples were re-vacuum packaged with Cryovac 872 AX.

#### Storage

Packs of bacon were held at 20 or 30°C and examined at 0, 2, 4, 8, 16 or 32 days for toxin assays.

#### Toxin analysis

The method of Pivnick and Bird (1965), was used. All bacon packs were tested for botulinum toxin and contents of each pack used for analysis.

#### Inactivation of toxin in bacon by heat

Samples of bacon with added *C. botulinum* toxin type B (80–160 MLD/g) were mixed and ground three times through a meat grinder. Aliquots of 10g samples were distributed into aluminum dishes (5 cm diam) and heated in ovens at 80, 89 and 103°C. Temperature of the ground bacon was measured by a telethermometer (Yellow Springs Instrument Co.) and recorded on a Honeywell Elektronik Recorder Model 194. At intervals, duplicate samples were removed and assayed for toxin. Heated samples with < 4 MLD/g were considered nontoxic.

#### Product acceptability

Bacon packs were stored at 30°C. After 0, 2, 4, 8, 16 and 32 days, packs were chosen at random and rated by a panel of three on the basis of acceptable or objectionable (putrid) odors. For presentation to the panel, the bacon was removed from the package, placed on a paper plate and covered with a plastic film. The film was partially removed during each evaluation.

### RESULTS & DISCUSSION

NITRITE, salt levels, moisture content and pH of bacon after processing are shown in Table 1. Losses of nitrite were up to one-third of the original levels. These data agree with those reported by Pivnick et al. (1967) and Greenberg (1972) for other cured products.

The formation of toxin by *C. botulinum* depended on the incubation temperature. Of 156 bacon packs with 0 to 200 ppm of NaNO<sub>2</sub> that were injected

Table 1—Analysis of side bacon after preparation and processing

Nitrite ppm		Salt			
Added	After processing	% (w/w)	% in water phase	Moisture % (w/w)	pH
0	4	2.0	6.4	29.3	6.1
50	32.0	1.8	5.7	30.0	6.0
100	68	1.7	5.1	31.4	6.2
150	104	1.6	4.9	31.1	6.3
200	144	1.8	5.2	32.7	6.2

Table 2—Toxigenesis of *C. botulinum* in vacuum-packed sliced bacon stored at 30°C

Nitrite level (ppm)	Inoculum <sup>a,b</sup> size (spores/g)	Experiment A <sup>c</sup> (Days)					Experiment B <sup>c</sup> (Days)			
		2	4	8	16	32	4	8	16	32
0	10 <sup>2</sup>	—	2/4	0/4	2/4	3/4	0/8	0/8	0/8	0/8
	10 <sup>4</sup>	0/4	0/4	1/4	3/4	3/4	1/8	0/8	5/8	6/8
50	10 <sup>2</sup>	—	0/4	1/4	1/4	1/4	0/8	0/8	0/8	1/8
	10 <sup>4</sup>	0/4	0/4	1/4	1/4	1/4	0/8	1/8	2/8	1/8
100	10 <sup>2</sup>	—	0/4	0/4	3/4	2/4	0/8	0/8	0/8	0/8
	10 <sup>4</sup>	0/4	0/4	2/4	2/4	2/4	0/8	2/8	0/8	2/8
150	10 <sup>2</sup>	—	—	1/4	0/4	1/4	0/8	0/8	0/8	0/8
	10 <sup>4</sup>	—	1/4	2/4	2/4	4/4	0/8	0/8	1/8	4/8
200	10 <sup>2</sup>	—	—	0/4	0/4	0/4	0/8	0/8	0/8	1/8
	10 <sup>4</sup>	—	0/4	0/4	0/4	3/4	1/8	0/8	0/8	0/8

<sup>a</sup> Bacon at all levels of nitrite inoculated with 10<sup>4</sup> spores/g was not toxic at day 0.  
<sup>b</sup> Inoculum contained two strains each of *C. botulinum* type A and B in approximately the same number.  
<sup>c</sup> Data are number of toxic packs/total number of packs tested; (—) not done.

with 10<sup>2</sup> spores/g and stored at 20°C, none was found to be toxic after 32 days. The equivalent data with 10<sup>4</sup> spores/g showed 1 pack with 100 ppm nitrite and 2 packs with 200 ppm nitrite toxic after 8 and 32 days, respectively, while none of the zero controls became toxic within 32 days.

In bacon stored at 30°C the incidence of toxic packs was considerably higher (Table 2). The results of experiment A indicate that at 10<sup>2</sup> spores/g there is some evidence (P = 0.06) that the number of toxic packs decreased with increasing nitrite content from 0–200 ppm. However, a relationship between nitrite levels and the prevention of toxin formation at 10<sup>4</sup> spores/g could not be established in this experiment (A). In experiment B (Table 2) the controls (0 ppm) at 10<sup>2</sup> spores/g did not become toxic so that no statistical data were obtained for this set of results. At 10<sup>4</sup> spores/g there was a significant decrease (P = 0.07) in toxic packs with increased nitrite concentration but no difference was found between 50 and 150 ppm. The variability of toxigenesis in the bacon samples (Table 2) might be explained by slight differences in salt and nitrite content between samples or by the indigenous microflora of the product (Pivnick and Bird, 1965).

The data in Table 2 demonstrate the effect of inoculum size on toxigenesis. A similar effect was observed by Steinke and Foster (1951) and Pivnick and Bird (1965).

Bacon packs of experiment B stored at 30°C were examined for off-odor and appearance before toxin analysis. The packs were selected at random throughout the storage period. The results are shown in Table 3. The panel accepted one-third of the toxic samples thus confirming the earlier findings of Greenberg et al. (1959) and Pivnick and Bird (1965)

that some meats could become toxic without becoming organoleptically offensive.

Figure 1 shows the inactivation of

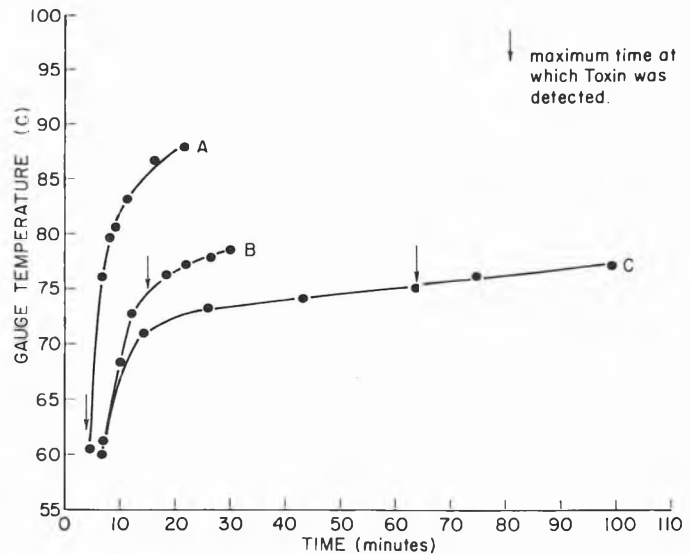


Fig. 1—Inactivation of *C. botulinum* toxin type A in bacon by heat: (A) oven temperature at 103°C; (B) oven temperature at 89°C; (C) oven temperature at 80°C.

Table 3—Acceptability<sup>a</sup> of toxic and nontoxic bacon packages incubated at 30°C

Observers	No. of toxic samples		No. of nontoxic samples	
	Accepted	Rejected	Accepted	Rejected
A	11	16	247	46
B	9	18	264	29
C	8	19	258	35

<sup>a</sup> Mean % samples accepted for nontoxic samples, 87.5; for toxic samples, 34.5.

toxin by heat. All samples became non-toxic when the bacon had reached the temperature of 75°C. During frying temperatures of 135–140°C are normally reached. However, in spite of this inactivation of toxin present in the bacon, consideration must be given to the possible transfer of toxin from the bacon to the consumer or other products before frying.

The experiments reported here show that both storage temperatures and spore level markedly affect the toxigenesis by *C. botulinum* in bacon and indicate that commercial bacon kept below 20°C is unlikely to become toxic. The results also show that toxigenesis can be reduced or delayed by addition of nitrite, particularly at a concentration of 200 ppm.

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## QUALITY ATTRIBUTES OF GROUND BEEF ON THE RETAIL MARKET

### INTRODUCTION

ACCORDING TO Federal definition, products labeled ground beef, chopped beef or hamburger may contain up to 30% fat by weight (Office of the Federal Register, 1973). Because of the wide allowance for fat, many retailers market ground beef at three or more lipid-price levels. The practice allows consumer choice, but also leads to confusion. Consumers should welcome research information concerning factors involved in the lipid-price relationship to aid in meat selection.

Results of research reported from consumer and laboratory taste panel studies that have attempted to define the most desirable lipid/lean ratio for ground beef have varied. Several researchers using consumer taste panels found that ground beef products containing 15–20% lipid were more acceptable than products higher (25–35%) in lipid content (Glover, 1964; Law et al., 1965; Carpenter and King, 1969; Mize, 1972). Conversely, Cole et al. (1960) reported that both laboratory and family taste panels rated ground beef containing 15% fat less palatable than ground beef containing 25, 35 or 45% fat; whereas, Nielsen et al. (1967), using a laboratory panel, and Kaiser et al. (1970), using a consumer taste panel, reported no significant differences in organoleptic scores for ground beef containing from 13–35% fat.

Cooking losses and their influence on cost per serving of ground beef products of various fat contents are another concern that needs to be defined. This study was designed to investigate palatability, cooking losses, other selected quality factors and cost per serving of three ground beef products purchased on the retail market.

### MATERIALS & METHODS

#### Purchasing and cooking

Three different formulations of packaged ground beef products were selected randomly from meat display cases of each of three local retail stores on 12 Mondays from Nov. 6, 1972 to March 5, 1973. Products from the three stores, representing a state chain, a local chain and a local Independent Grocers' (IGA) affiliate, were used for the first 6 wk (replications). Because the IGA changed ownership after the

sixth week, only two stores were represented in the final six replications. After being purchased, the products were divided randomly into two groups, and held in a refrigerator at 3°C until cooked and evaluated Tuesday and Wednesday afternoons.

Three 180-g patties, approximately 2.5 × 7.6 × 8.2 cm were molded from each ground beef product, placed side by side on a wire rack 23 cm high, set in a shallow pan and cooked in a rotary hearth gas oven maintained at 177°C for 35 min.

#### Internal temperature, cooking losses and cost per 100-g serving

At the end of the cooking period, the temperature reached in the middle patty on the rack was recorded, and percentages of total, volatile and drip cooking losses, based on the weight of the three raw patties, were calculated. Cost per 100-g serving of cooked meat was calculated by dividing the price of 100g of raw meat by percentage yield (1.0–percentage total cooking losses). For objective and sensory evaluation, the crust of each patty was re-

Table 1—Product labeling, ether extract and mean price per pound for ground beef from three retail stores

Product	Store		
	A State chain	B Local chain	C Local IGA
<b>1</b>			
Label	Regular Approx 73% lean	Regular 70–75% lean	Regular —
Ether extract, %			
6 wk <sup>a</sup>	30.8	29.1	22.1
12 wk <sup>b</sup>	31.0	28.0	—
Mean price/lb, \$			
6 wk <sup>a</sup>	0.70	0.70	0.74
12 wk <sup>b</sup>	0.73	0.73	—
<b>2</b>			
Label	Lean Approx 80% lean	Lean 75–80% lean	Ground chuck not less than 90% lean
Ether extract, %			
6 wk <sup>a</sup>	26.8	24.4	8.8
12 wk <sup>b</sup>	26.0	23.2	—
Mean price/lb, \$			
6 wk <sup>a</sup>	0.80	0.80	0.96
12 wk <sup>b</sup>	0.84	0.84	—
<b>3</b>			
Label	"Diet" lean Approx 88% lean	Extra lean 80–85% lean	Ground round not less than 90% lean
Ether extract, %			
6 wk <sup>a</sup>	9.4	11.4	9.9
12 wk <sup>b</sup>	11.2	12.2	—
Mean price/lb, \$			
6 wk <sup>a</sup>	1.00	1.00	1.06
12 wk <sup>b</sup>	1.04	1.04	—

<sup>a</sup> Observations between Nov. 6, 1972 and Jan. 6, 1973

<sup>b</sup> Observations between Nov. 6, 1972 and March 5, 1973



moved; the patty with the recorded internal temperature was evaluated for palatability, and the other two were used for objective measurements.

#### Sensory evaluation

Cubes (1.9 cm), cut from the designated cooked patty, were placed in ceramic casseroles, and held at constant low heat ( $35^{\circ} \pm 1^{\circ}\text{C}$ )

on an electric hot tray for evaluation by a 7-member "experienced" laboratory panel within 30 min.

Panel members randomly selected cubes from the casseroles and evaluated them using a 5 to 1 scale. Flavor and over-all acceptability were scored for desirability (5 = extremely desirable to 1 = undesirable), juiciness was scored for intensity (5 = extremely juicy to 1 =

dry) and texture was scored for type (5 = mealy to 1 = chewy).

Ether extract, total moisture, press fluid and depth of penetration

Percentages of ether extract in both raw and cooked products were measured according to a modification of the AOAC method (AOAC, 1970). Duplicate 2.5-g samples were dried in 11.5-g teflon coated pans for 60 min at  $121^{\circ}\text{C}$  in a C.W. Brabender Semi-automatic Rapid Moisture Tester. Dried samples were rolled in Whatman No. 5 filter paper and transferred to extraction thimbles. Pans used for drying were rinsed with petroleum ether used in the extraction process to include lipid lost during drying. Samples were extracted for 16 hr on a Goldfisch extraction apparatus, the ether was evaporated and percentage ether extract was calculated.

Percentage total moisture for each product was determined by drying duplicate 10-g samples of ground, cooked meat in a C.W. Brabender Semi-automatic Rapid Moisture Tester for 60 min at  $121^{\circ}\text{C}$ .

Press fluid from each product was measured on duplicate 25-g samples of ground, cooked meat packed in a cheesecloth-lined (2 layers, 14.5 cm diam) cylinder of a Carver Laboratory Press. The sample was divided roughly into thirds, and packed in the cylinder by alternating the meat with four circles (5.5 cm) of Whatman No. 1 filter paper. The packed cylinder was pressed following a standardized 15-min, time-pressure schedule with maximum pressure of 4,000 psig. The expressed fluid was poured into centrifuge tubes graduated in 0.1 ml, capped with aluminum foil and placed in a refrigerator until the following day when the volumes of total press fluid, serum, fat and solids were read.

The depth (mm) of penetration into one crustless patty from each product was measured with a Universal Precision Penetrometer at five locations (mid-point, mid-left, mid-right, mid-upper, mid-lower). Readings obtained from allowing the weight of the penetrometer's cone (150g) to drop into the meat for 5 sec at each location were averaged to determine depth of penetration.

#### Statistical analyses

Data obtained from each evaluation measurement were analyzed by analysis of variance. Because products from one store were eliminated the last 6 wk, data for the first 6 wk (three products, three stores) were analyzed as one experiment. Data for 12 wk for the same three products from two stores were analyzed as a second experiment. For each source of variation for which the F-value was significant, least significant difference at the 5% level of probability was calculated. Bartlett's test for homogeneity of variance was used to study variation among weeks for a given product; also, Bartlett's test was used to estimate variance among the nine product-store combinations. A regression equation was derived to study the relationship between percentage yield of cooked ground beef and percentage lipid in the raw meat.

## RESULTS & DISCUSSION

### Product label and price differences among stores

Labeling terminology and mean price per lb during 6- and 12-wk periods for the

Table 2—Means, significance of F-ratios and LSD attributable to products and stores for objective and subjective measurements of ground beef

Measurement	Weeks <sup>a</sup>	Product <sup>b</sup>			Signifi- cance of F	LSD <sup>d</sup>	Store <sup>c</sup>			Signifi- cance of F	LSD <sup>d</sup>
		1	2	3			A	B	C		
Ether extract, %											
Raw meat	6	27.4	20.0	10.2	***	2.3	22.4	21.6	13.6	***	2.3
	12	29.5	24.6	11.7	***	2.2	22.7	21.1	—	ns	—
Cooked meat	6	19.0	15.6	12.1	***	1.8	16.9	16.2	13.4	**	1.8
	12	19.6	17.7	12.1	***	1.5	16.6	16.3	—	ns	—
Cooking losses, %											
Total	6	29.9	25.5	20.4	***	2.1	27.1	27.3	21.3	***	2.1
	12	31.6	28.7	20.4	***	1.8	27.3	26.5	—	ns	—
Volatile	6	16.5	16.5	17.6	*	1.0	16.7	17.0	16.8	ns	—
	12	16.4	16.8	17.4	*	0.8	16.6	17.1	—	ns	—
Drip	6	12.9	8.6	2.5	***	1.7	10.0	10.0	4.1	***	1.7
	12	14.8	11.0	2.6	***	1.6	10.1	8.9	—	ns	—
Cost/100-g serving, cents											
	6	22.4	25.3	28.2	***	0.7	25.1	25.1	25.7	ns	—
	12	23.5	25.8	28.7	***	0.6	26.1	25.8	—	ns	—
Internal temp, °C											
	6	69.9	68.3	66.2	**	2.1	69.3	68.3	66.8	ns	—
	12	70.4	69.4	66.8	***	1.7	69.9	67.9	—	**	1.4
Total moisture, %											
	6	56.5	60.1	62.7	***	1.8	58.5	59.0	61.7	**	1.8
	12	56.1	58.2	63.0	***	1.5	58.8	59.4	—	ns	—
Press fluid, ml/25g											
Total	6	7.2	7.4	7.1	ns	—	7.6	7.0	7.1	**	0.3
	12	7.2	7.3	7.4	ns	—	7.4	7.2	—	***	0.1
Serum	6	4.5	5.0	5.3	*	0.6	5.0	4.6	5.1	ns	—
	12	4.2	4.6	5.7	***	0.3	5.0	4.7	—	*	0.3
Fat	6	2.0	1.6	0.9	***	0.4	1.9	1.4	1.2	**	0.4
	12	2.2	2.0	1.0	***	0.3	1.9	1.6	—	*	0.3
Solids	6	0.7	0.9	0.9	ns	—	0.6	1.0	0.9	ns	—
	12	0.8	0.7	0.6	ns	—	0.6	0.9	—	*	0.2
Penetration, mm											
	6	13.5	13.2	12.2	ns	—	12.3	13.3	13.2	ns	—
	12	13.4	13.6	13.1	ns	—	12.8	13.9	—	**	0.7
Sensory scores <sup>e</sup> , 5-1											
Flavor	6	3.3	3.2	2.9	ns	—	3.0	3.3	3.2	ns	—
	12	3.3	3.3	2.9	**	0.3	3.0	3.3	—	ns	—
Texture	6	3.0	3.1	3.5	ns	—	3.1	3.2	3.3	ns	—
	12	3.0	3.1	3.5	*	0.4	3.1	3.3	—	ns	—
Juiciness	6	3.4	3.2	3.0	ns	—	3.1	3.3	3.2	ns	—
	12	3.4	3.2	2.9	*	0.3	3.1	3.2	—	ns	—
Over-all acceptability											
	6	3.2	3.1	3.0	ns	—	3.0	3.2	3.1	ns	—
	12	3.3	3.2	3.0	ns	—	3.1	3.3	—	ns	—

<sup>a</sup> Values for 6 wk are for three products, three stores; values for 12 wk are for three products, two stores (A and B).

<sup>b</sup> Values for products 1 (lowest-priced), 2 (medium-priced) or 3 (highest-priced) from all stores combined

<sup>c</sup> Values for all products from A (state chain), B (local chain) or C (local IGA)

<sup>d</sup> Least significant difference at 5% level

<sup>e</sup> Range: 5 = extremely desirable, extremely juicy or mealy; 1 = undesirable, dry or chewy

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

products evaluated are presented in Table 1. Except for products 2 and 3 from store C, an increase in price per pound between any two ground beef products was accompanied by an increase in the labeled percentage of lean meat.

The stores differed slightly in labels and prices given each of their products. Stores A and B used descriptive and percentages of lean statements to identify the type of product. Store C did not use the percentage lean on its label for regular ground beef, product (1C), and labeled products 2 and 3 according to primal cut (chuck and round) and percentage lean.

#### Percentage ether extract

Mean values for percentage lipid (ether extract) in raw products (Table 1) showed that stores C and B stayed within federal regulations and their own labeling statements concerning the fat content in their products, except that store B product 2 averaged 3–4% above the labeled percentage nonlean (Table 1). Products 1 and 2 from store A averaged approximately 4.0–7.0%, respectively, above labeled approximate percentage nonlean (Table 1), and 1A averaged 1.0% above federal regulations for the maximum amount of fat allowable in ground beef (30%).

Mean values for objective and subjective measurements attributable to products (all stores combined) and to stores (all products from one store combined) are given in Table 2. The values for 6 wk were derived from an analysis of data for three products, three stores; whereas, the

values for 12 wk were derived from an analysis of data for three products, two stores (A and B). As product number and price per pound increased, percentage ether extract in both raw and cooked products decreased ( $P < 0.05$ ). However, differences in ether extractable material between products 1 and 3 were not as great for cooked meat as for raw meat, because percentage ether extract was less (3.7–10.7%) after cooking for product 1 and for product 2 from stores A and B, and slightly greater after cooking (0.3 to 2.4%) for product 2 from store C and for product 3 (Table 2). Product 3 contained more ( $P < 0.05$ ) moisture and had greater ( $P < 0.05$ ) volatile losses, but less ( $P < 0.05$ ) drip cooking losses than products 1 and 2 (Table 2). That may account, partially, for the small increase in ether extract of the cooked product. Irmiter et al. (1967) also found that percentage ether extract decreased with cooking in ground beef containing 30% lipid and increased with cooking in ground beef with 3–20% lipid.

Woolsey and Paul (1969) reported that both petroleum ether (nonpolar solvent) and chloroform-methanol (polar solvent) extracted significantly more crude fat from cooked than from raw, lean, intact semitendinosus muscle, even when results were calculated on a dry weight basis. Moreover, differences in the amount of fat extracted by the two solvents were not significant. In their study, they concluded that heating caused denaturation of protein and subsequent release of lipid previously complexed with protein so

that lipid was more accessible to both polar and nonpolar solvent extraction. They also suggested that the slow increase in temperature at the beginning of the cooking process may have activated enzymes, which in turn released bound fat.

For both raw and cooked products, percentage ether extract from store C products averaged lower ( $P < 0.05$ ) than that from either store A or store B products (Table 2).

Significant product  $\times$  store interactions found with analysis of the data for the 6- and 12-wk periods are presented in Table 3. Percentage ether extract was lower ( $P < 0.05$ ) in regular ground beef from store C (1C) than in regular ground beef from store A or B (1A, 1B), or in lean ground beef from store A (2A). Also, product 2 from store C (ground chuck) averaged lower ( $P < 0.05$ ) in ether extractable material than product 2 from store A or B (ground lean), and was similar in ether extract to product 3 from all stores.

#### Cooking losses and cost per serving

As product number and price per pound increased, total and drip cooking losses decreased ( $P < 0.05$ ). However, volatile cooking losses were greater ( $P < 0.05$ ) for the highest-priced product (3) than for the lowest-priced product (1) for the 6- and 12-wk periods, and greater ( $P < 0.05$ ) than for the medium-priced product (2) for the 6-wk period. Total and drip cooking losses were greater ( $P < 0.05$ ) for both store A and store B products than for store C products, but mean values for volatile cooking losses varied little among stores (Table 2).

Significant ( $P < 0.05$ ) 6-wk product  $\times$  store interactions for total and drip cooking losses occurred that were similar to the 6-wk product  $\times$  store interaction for ether extract in raw products (Table 3). Product 1C (regular) had less ( $P < 0.05$ ) total and drip cooking losses than 1A or 1B (regular) and less ( $P < 0.05$ ) drip cooking losses than 2A or 2B (ground lean). Also, total and drip cooking losses from 2C (ground chuck) were less ( $P < 0.05$ ) than those from 2A or 2B (ground lean) and were similar to those of 3A, 3B or 3C ("diet" lean, extra lean, ground round).

Although total cooking losses were less for leaner, higher-priced products, the increases in price per pound for those products were such that cost per 100-g serving of cooked meat increased ( $P < 0.05$ ) as lean (product number) increased (Tables 1 and 2). During the first 6 wk, store C products cost an average of 9 cents more per pound (Table 1), but had 5.8 and 6.0% lower total cooking losses (Table 2) than store A and store B products, so the cost per 100g of cooked meat did not vary significantly among stores (Table 2).

Table 3—Significant product  $\times$  store interactions

Measurement	Weeks <sup>a</sup>	Product <sup>b</sup>	Store <sup>c,d</sup>			Significance of F	LSD <sup>e</sup>
			A	B	C		
Ether extract, raw meat, %	6	1	30.8d	29.1d	22.1f	***	4.0
		2	26.8de	24.4ef	8.8g		
		3	9.4g	11.3g	9.9g		
Total cooking losses, %	6	1	32.1d	31.4d	26.1e	**	3.7
		2	28.6de	29.6de	18.1f		
		3	20.4f	20.8f	19.8f		
Drip cooking losses, %	6	1	15.3d	15.3d	8.3f	***	3.0
		2	12.0e	11.7e	2.0g		
		3	2.6g	2.9g	2.1g		
Total press fluid, ml/25g	12	1	7.2de	7.2de	—	**	0.3
		2	7.4e	7.3de	—		
		3	7.7f	7.0d	—		
Serum in press fluid, ml/25g	12	1	4.2d	4.3de	—	*	0.4
		2	4.7e	4.5de	—		
		3	6.1g	5.4f	—		

<sup>a</sup> Weeks, 6 (three products, three stores); 12 (three products, two stores, A and B)

<sup>b</sup> Product, 1 (lowest-priced); 2 (medium-priced); 3 (highest-priced)

<sup>c</sup> Store, A (state chain); B (local chain); C (local IGA)

<sup>d</sup> Means for a measurement bearing the same letter do not differ significantly at 5% level.

<sup>e</sup> LSD, least significant difference at 5% level

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

A regression equation (Fig. 1,  $a + \beta x + \epsilon$ ) based on 36 observations from each of stores A and B and 18 observations from store C was used to evaluate the relationship between percentage yield of cooked meat (Y) to percentage lipid (X), (ether extract) in raw meat. The estimated regression equation was  $\hat{y} = 85.4 - 0.55x$  with a standard deviation of 3.2 and  $r^2 = 0.70$ . Assuming cooking conditions elsewhere are similar to those in this study, the regression equation may be used to calculate the cost per pound, or per serving, of cooked meat given percentage lipid and price per pound for the raw meat. For example, at \$1.00 per pound for regular ground beef (approx 30% lipid), a pound of cooked meat would cost \$1.45; at \$1.10 per pound for lean ground beef (approx 20% lipid) a pound of cooked meat would cost \$1.48; and at \$1.20 per pound for extra lean ground

beef (approx 10% lipid) a pound of cooked meat would cost \$1.50.

Internal temperature, total moisture, press fluid and depth of penetration

After 35 min cooking at 177°C, the internal temperature was higher ( $P < 0.05$ ) for the two higher-lipid products (1 and 2) than for the leaner product (3), Table 2. That agrees with findings of Irmiter et al. (1967) and Funk and Boyle (1972) that products with the lowest lipid content, in ground beef cylinders containing from 2–30% lipid, required the longest time to reach 80°C.

During the first 6 wk, differences in internal temperature of products were not significant among stores, but with data for 12 wk, the internal temperature of store A cooked products was significantly higher than that of store B cooked products (Table 2).

As expected, percentage total moisture in cooked products increased ( $P < 0.05$ ) as lipid content decreased and product number increased, indicating that total moisture was inversely related to lipid content. Also, products from store C were higher ( $P < 0.05$ ) in total moisture than products from store A or store B (Table 2).

Total press fluid did not vary significantly among products; however, mean total press fluid was higher ( $P < 0.05$ ) for store A products than for store B or store C products (Table 2). While standing, press fluid separated into fat, serum and solids. As the lipid content of the product increased, press fluid contained less serum and more separable fat (Table 2). Also, press fluid from store A products contained more ( $P < 0.05$ ) serum than press fluid from store B products (12 wk), and more separable fat ( $P < 0.05$ ) than press fluids from store B (6 and 12 wk) or store C (6 wk) products. During the first 6 wk, solids in press fluid did not vary significantly among products or stores, but analysis of data for 12 wk showed that store B products had more ( $P < 0.05$ ) solids in press fluid than did store A products (Table 2).

Depth of penetration into cooked meat by a cone (weight 150g) on the Universal Precision Penetrometer did not

Table 4—Variances that differed ( $P < 0.05$ ) among product-store combinations

Measurement	Product-store <sup>a</sup>	Variance <sup>b</sup>	Measurement	Product-store <sup>a</sup>	Variance <sup>b</sup>
Total cooking losses, %	3C	2.12a	Drip cooking losses, %	3C	0.21a
	1C	3.41a		2C	0.41ab
	2C	4.03a		3B	1.58bc
	3A	4.03a		3A	2.67c
	3B	4.38ab		1C	2.76c
	1A	10.46bc		1A	10.41d
	2A	13.74c		2A	12.05d
	1B	14.66c		1B	13.85d
	2B	18.91c		2B	14.29d
	Fat in press fluid, ml/25g	2C		0.08a	Solids in press fluid, ml/25g
3C		0.09a	3B	0.26ab	
2A		0.20ab	1B	0.40abc	
1A		0.21ab	3A	0.50abc	
3A		0.31abc	1A	0.55abc	
1B		0.39abc	2C	0.57abc	
3B		0.39abc	3C	0.67bc	
1C		0.53bc	1C	1.63c	
2B		1.27c	2B	1.75c	
Flavor, 5-1		1C	0.07a	Juiciness, 5-1	
	2C	0.09a	3C		0.13b
	3B	0.09a	1C		0.23bc
	1A	0.09a	2A		0.25bc
	1B	0.09a	3A		0.28bc
	2A	0.16a	1A		0.38bc
	2B	0.20ab	2C		0.38bc
	3C	0.36b	1B		0.43bc
	3A	0.53b	2B		0.51c
	Over-all acceptability, 5-1	1C	0.05a		
2C		0.05a			
3B		0.05a			
3C		0.10ab			
1A		0.11ab			
2A		0.23bc			
	2B	0.25bc			
	3A	0.56c			

<sup>a</sup> Product-store, 1 (lowest-priced), 2 (medium-priced) and 3 (highest-priced) products from A (state chain), B (local chain) or C (local IGA)

<sup>b</sup> Variances for the same measurement bearing the same letters are not significantly different ( $P < 0.05$ ).

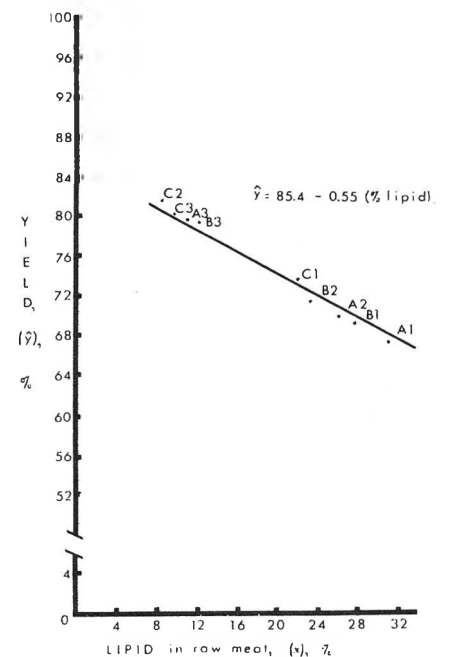


Fig. 1—Regression equation for percentage yield of cooked meat vs. percentage ether extract (lipid) in raw meat. The regression line was calculated from data for 90 observations; the graph represents the average of each store-product.

vary significantly among products or among stores for the 6-wk period, but for the 12-wk period depth of penetration into store B products was greater ( $P < 0.05$ ) than that into store A products (Table 2).

#### Sensory evaluation

For the first 6 wk, scores for flavor, juiciness and over-all acceptability did not vary significantly among products or stores. For the 12-wk period, over-all acceptability did not vary significantly among products or stores, and flavor, juiciness and texture did not vary among stores. However, for the 12-wk period, the two higher-lipid, lower-priced products (1 and 2) rated better ( $P < 0.05$ ) in flavor and chewier ( $P < 0.05$ ) in texture than the leanest, highest-priced products (3), and the highest-lipid product (1) rated juicier ( $P < 0.05$ ) than the leanest product (3) (Table 2).

#### Variation among weeks, week $\times$ product interactions and week $\times$ store interactions

Lipid, moisture, total and drip cooking loss and palatability data were similar from week-to-week for the ground beef products. For the first 6 wk, significant ( $P < 0.05$ ) differences attributable to the week in which products were purchased occurred only for volatile cooking losses, total press fluid and solids in the press fluid. During the 12-wk period, differences ( $P < 0.05$ ) attributable to the week purchased also occurred for internal temperature, depth of penetration, fat in press fluid and cost per 100-g serving. Differences among weeks in cost per 100-g serving are the only one of those measurements that can be explained. The cost per pound of all products from all stores was constant for weeks 1 through 5; then, on weeks 6 through 12, increases in cost per pound occurred for one or more products each week except one. That increased ( $P < 0.05$ ) the cost per 100-g serving of cooked meat.

Significant product  $\times$  week interactions did not occur for any measurement during the first 6 wk, and occurred only for total press fluids and for serum and solids in the press fluids during the 12-wk period. The only significant ( $P < 0.05$ ) week  $\times$  store interactions that occurred

were for drip cooking losses during both the 6- and 12-wk period and for total press fluids and fat in the press fluids during the 12-wk period. None of those interactions seemed important.

#### Variance among products

Law et al. (1965) reported that consumers they surveyed stated that they consistently purchased "ground round" or "ground chuck," because they could be assured of greater uniformity than if they purchased "ground beef." In this study, variance among the nine product-store combinations was estimated for each measurement by subjecting data to Bartlett's test for homogeneity of variance. Variances for the seven measurements that differed ( $P < 0.05$ ) among the nine product-store combinations are given in Table 4.

Total and drip cooking loss variances were lowest ( $P < 0.05$ ) for leaner products 3C (ground round), 2C (ground chuck), 3A ("diet" lean), 3B (extra lean) and for 1C (regular); they were greatest ( $P < 0.05$ ) for higher-lipid products 1A and 1B (regular) and 2A and 2B (lean). Variances for the amount of fat in press fluids were lowest for 2C and 3C (ground chuck and ground round), but those variances were not significantly less than variances for products 1, 2 and 3 from store A and products 1 and 3 from store B. Variances for solids in press fluid, flavor, juiciness and over-all acceptability also differed ( $P < 0.05$ ) among product-store combinations (Table 4), but trends were not apparent.

### CONCLUSIONS

UNDER the conditions of this study, it was concluded that:

1. Ground beef that contains 10–20% lipid has less cooking loss than ground beef that contains 25–30% lipid.
2. In general, percentage ether extract (lipid) decreases with broiling when raw ground beef contains 20–30% lipid, and increases slightly with broiling when the raw meat contains less than 12% lipid; therefore, the difference in lipid content between the above two classifications of ground beef products is less for broiled than for raw meat.

3. Lower-lipid, higher-priced ground beef cost more per 100g cooked meat than higher-lipid lower-priced beef.
4. The ratio of lipid/lean has no significant effect on over-all acceptability of ground beef products, but the leanest products usually available (9–12% lipid) tend to be less juicy, more mealy in texture and less desirable in flavor than products higher in lipid content.

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## FREEZING OF COOKED MEAT: INFLUENCE OF FREEZING RATE AND RECONSTITUTION METHOD ON QUALITY AND YIELD

### INTRODUCTION

THE GROWING importance of frozen minced or finely cut meats (raw or pre-cooked) on the consumer market has created a need for more knowledge on the combined influence on sensory quality and yield of factors such as freezing rate, frozen storage conditions and reconstitution methods. In previous papers by the authors (Jakobsson and Bengtsson, 1969; 1973) work was reported for raw minced and sliced beef. The present paper concerns work with precooked meats.

Only one report was found in the literature dealing specifically with effects of freezing rate on the quality of cooked beef in small cuts, and thus up to high freezing rates. Andreotti and Ferlinghi (1967) found better retention of sensory quality in small cuts of cooked beef and less juice loss when freezing by liquid nitrogen spray than by conventional blast freezing. In a preliminary study with pan-fried meat patties, Jakobsson and Bengtsson (1969) found a slight improvement in final yield with increasing freezing rate. As to the influence of frozen storage, literature data on practical shelf life of precooked foods without gravy vary in the range 250–900 days at  $-20^{\circ}\text{C}$ , with a discernible sensory change within one-third of this time, Bengtsson et al. (1972).

In a comparison of reheating methods for frozen precooked foods, Causey and Fenton (1951) found ham patties to be

more palatable when thawed before being reheated. A comparison between reheating in a convection oven, household oven, boil-in-bag and microwave oven showed the greatest loss and lowest palatability for microwave heating under the experimental conditions used. But, in the study by Jakobsson and Bengtsson (1969) precooked meat patties were reheated directly from the frozen state by pan frying and by continuous microwave heating without any clear quality differences between the reconstitution methods used.

### EXPERIMENTAL

#### Experimental plan

The investigation comprised two separate experiments, one with sliced beef and the other with meat patties. The raw materials used and variables studied are summarized in Table 1. In the experiments the effect of a wide range of freezing rates were studied, using two different heating methods for reconstitution from both the frozen and the thawed state after frozen storage. (The design of the  $4 \times 2 \times 2 \times 2$  and  $4 \times 3 \times 2 \times 2$  factorial experiments on sliced beef and patties is apparent from Tables 3 and 5.)

#### Raw material and preparation

The composition before and after cooking of the raw materials used in the cooking and freezing experiments is given in Table 2.

In experiment No. 1, 1.5 cm slices of longissimus (LD) muscle from the hind part (from the 11th vertebra) of nine 2-yr-old steers of 290 kg slaughtered weight were used. Each muscle yielded about 20 slices, which were assigned to the variables studied in a systematic manner to

ensure that comparisons for a given property would be made between equivalent groups of slices. Slices were pan fried to  $70-75^{\circ}\text{C}$  central temperature prior to freezing, using a pan temperature of  $175^{\circ}\text{C}$ .

In experiment No. 2, meat patties of 70g raw weight of a commercial recipe were deep-fat fried at  $160^{\circ}\text{C}$  for 2.5 min to a final central temperature of  $70^{\circ}\text{C}$ .

#### Freezing

Freezing to a final central temperature of  $-25$  to  $-30^{\circ}\text{C}$  was started within 5 min of pre-cooking, except for one variable in which samples were first cooled before being packed and pallet frozen.

Immersion freezing in liquid nitrogen ( $\text{LN}_2$ ) was pulsed for sliced beef to prevent surface cracking. Total treatment time was 1.3 min and freezing rate was around 200 cm/hr, expressed as half the thickness of the sample in cm divided by the time in hours required to lower the central temperature from  $0^{\circ}\text{C}$  to  $-10^{\circ}\text{C}$ . Meat patties were frozen in 55 sec using constant immersion, at about the same freezing rate.

Spray freezing with  $\text{LN}_2$  was performed in a continuous pilot freezer of our design in which total treatment time, excluding precooling but including equilibration zones, was 10 min. Freezing rate was around 20 cm/hr.

Air blast freezing was done in a pilot blast freezer at  $-35^{\circ}\text{C}$  giving a total freezing time of 45 min and freezing rate of 3 cm/hr.

Simulated in-package pallet freezing was performed in a well insulated box inside a  $-20^{\circ}\text{C}$  freezer, giving a freezing rate below 0.1 cm/hr.

#### Packaging, frozen storage and reconstitution

In all experiments, flexible pouches of good

Table 1—Raw materials and variables studied in experiments

Exp. No.	Raw material	Variables studied	Precooking method	Freezing method	Storage	Reconstitution
1	1.5 cm slices longissimus	Freezing rate, frozen storage conditions, reconstitution	Pan frying at $175^{\circ}\text{C}$ 4 min either side	Pulsed $\text{LN}_2$ -immersion, $\text{LN}_2$ -spray, air blast at $-30^{\circ}\text{C}$ , simulated in-package pallet freezing	$-20^{\circ}\text{C}$ 2 and 7 months nitrogen headspace	Pan frying and microwave tunnel from $+5^{\circ}$ and $-20^{\circ}\text{C}$
2	70g meat patties (1.0 cm thickness)	Freezing rate, frozen storage conditions, reconstitution	Deep-fat frying 2.5 min at $160^{\circ}\text{C}$	Pulsed $\text{LN}_2$ -immersion, $\text{LN}_2$ -spray, air blast at $-30^{\circ}\text{C}$ , simulated in-package pallet freezing	$-20^{\circ}\text{C}$ 2 and 7 months air and nitrogen headspace	Pan frying and microwave tunnel from $+5^{\circ}$ and $-20^{\circ}\text{C}$

barrier properties were used (saran-coated cellophane/polyethylene) with as little air or nitrogen headspace as possible. The samples were stored at  $-20^{\circ}\text{C}$  for periods of a few days to 7 months. The samples were either thawed at room temperature to a central temperature of  $+5^{\circ}\text{C}$  prior to reheating or heated directly from the frozen state to a central temperature of  $60^{\circ}\text{C}$ . They were pan fried at  $160^{\circ}\text{C}$  in margarine, turning the sample over every 2 min. Reheating by microwaves was done continuously in a 5 kW Husqvarna wave-guide tunnel at 2450 MHz inside small plastic boxes.

#### Analytical methods

Sensory evaluation was made using 9-grade intensity scales for off-flavor, flavor, juiciness,

Table 2—Raw material composition

	Exp No. 1 Sliced beef	Exp No. 2 Meat patties
Meat content, %	100	70
Proportion pork:beef	—	1:1
Potato base "filler," %	—	25
Milk protein and starch- base binder, %	—	2
Water content, raw %	72–75	59
Fat content, raw %	2–5	12
Water content, fried %	60	55.5
Fat content, fried %	5	12

tenderness or texture goodness and appearance (1 = no off-flavor, extremely poor, dry, tough or good; and 9 = extremely high off-flavor or extremely good, juicy tender etc.). The four to five member panel was chosen from people with previous experience in evaluating cooked meat and trained on the specific materials and techniques used in these experiments. In all sessions a reference stored at  $-80^{\circ}\text{C}$  was included and its rating was determined at each session as a means of "calibrating" the panel. The references were thawed before reheating. Throughout the testing they were of a slightly higher quality than the samples.

TBA-test as a measure of rancidity was performed before reheating and according to Tar-

Table 3—Precooked, sliced beef. Influence of freezing rate, frozen storage, reconstitution method and initial sample temperature.

#### 1. Source of variation, degrees of freedom, mean squares and level of statistical significance

Source of variation	dF	Mean squares					
		Flavor	Off flavor	Juiciness	Tenderness	Reconstitution loss	Centrifugation loss
Freezing rate (F)	3	3.073	7.256**	0.356	0.333	19.89**	977.8
Storage time (S)	1	16.26	1.806	14.70	57.60*	5.377	14002**
Reheating method (R)	1	0.506	10.51*	66.31	3.03	524.9**	—
Initial temp (I)	1	1.056	0.156	4.56	15.63	37.41	—
F X S	3	0.856	0.873	1.056	1.367	2.00	1361.5
F X R	3	2.21	1.873	0.290	0.158	2.041	—
F X I	3	1.623	0.890	0.440	1.492	3.759	—
S X R	1	13.81**	3.906	19.756**	0.625	43.25**	—
S X I	1	3.306	1.056	0.756	14.93***	16.55*	—
R X I	1	2.756	3.306	0.506	0.0001	12.25*	—
Error MS		1.505	1.213	1.979	1.0026	2.96	1215.8
Error dF		141	141	141	141	13	24

#### 2. Mean values, number of observations and S.E. of means<sup>a</sup>

Treatment	Flavor score	Off-flavor score	Juiciness score	Tenderness score	Reconstitution loss, %	Centrifugation loss, %
Freezing rate:						
200 cm/hr	4.4	2.2b	3.9	6.4	9.6a	25.4
20 cm/hr	4.9	1.3a,c	4.0	6.2	9.1a	22.9
3 cm/hr	4.7	1.7c	3.8	6.3	11.6a,b	24.1
0.1 cm/hr	4.3	2.2b	3.9	6.2	12.7b	23.8
No. of observations	40	40	40	40	8	8
S.E.	0.19	0.17	0.22	0.16	0.61	12.33
Frozen storage:						
2m, $-20^{\circ}\text{C}$	5.0	1.9	4.4	5.7	10.0	20.2
7m, $-20^{\circ}\text{C}$	4.1	1.7	3.5	6.9	11.4	25.3
No. of observations	80	80	80	80	16	16
S.E.		0.12				8.72
Reheating method:						
Pan frying	4.8	1.6	3.6	6.1	14.0	
Microwave	4.4	2.1	4.2	6.4	7.6	
No. of observations	80	80	80	80	16	
S.E.		0.12		0.11		
Initial product temp in reheating:						
Thawed	4.8	1.8	4.1	6.0	9.6	
Frozen	4.6	1.9	3.7	6.6	12.0	
No. of observations	80	80	80	80	16	
S.E.	0.14	0.12	0.16			

<sup>a</sup> Where more than two means are compared, different index letter signifies that values are significantly different at least at  $P < 0.05$ .

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

ladgis et al. (1960) measuring extinction at 530 nm.

Percent centrifugation loss was used as a measure of water-holding capacity for the thawed, cooked meat. Samples of 10g of meat patties were wrapped in nylon cloth, placed on a perforated disc on a 55 mm diameter holder, and centrifuged at 1200g for 15 min, while 2-g samples of whole beef were centrifuged according to a method by Aitken et al. (1962).

Reconstitution loss (thawing and reheating losses combined) as a measure of yield were determined and expressed as a percentage of the frozen sample weight.

**RESULTS & DISCUSSION**

THE COMBINED EFFECTS of freezing rate, frozen storage and the method and initial sample temperature of reconstitution for cooked sliced or minced meats are shown in Tables 3-5.

**Freezing of cooked sliced beef (Tables 3-4)**

Significant interactions were found between frozen storage time at -20°C and reconstitution method for flavor, juiciness and yield, between frozen storage time and initial temperature of reconstitution for tenderness and yield, and between reconstitution method and initial temperature for yield.

Overriding these interactions, significant differences were found between freezing rates, storage times and reconstitution methods for one or more quality aspects. Because of the number of interactions obtained, separate analyses of variance for flavor, juiciness, tenderness and yield were run as summarized in Table 4. In some cases separation down to two-factor analysis was necessary to clarify the nature of differences between variable levels.

The two extreme freezing rates gave significantly higher off-flavor scores than the others. Yield increased with the freezing rate, but sensory quality retention did not improve significantly.

With prolonged frozen storage at -20°C tenderness increased significantly and water holding ability (as measured by centrifugation loss) decreased.

In the complete analysis of variance in Table 3, tendencies to decreasing flavor and juiciness scores with increasing frozen storage time were masked by interactions between storage time and method of reconstitution. In a separate analysis of variance for reconstitution by pan frying (Table 4) these tendencies became significant, particularly for pan frying after previous thawing, when also the yield was

found to decrease significantly with prolonged frozen storage. Microwave heating showed less quality change with storage time than did pan frying.

With regard to reconstitution method (Table 3), a significant advantage in yield for reconstitution by microwave over pan frying was accompanied by a tendency towards higher juiciness and lower flavor scores for microwave. Further separation of the ANOVA (see Table 4) revealed that the flavor disadvantage was significant for the shorter storage time. As already indicated, microwave heating showed less quality change with frozen storage time than did pan frying.

Overall tendencies in the main ANOVA toward higher tenderness and lower yield for heating directly from the frozen condition became significant for the short storage time. The negative in yield was more evident when reheating by microwaves than by pan frying. The flavor difference between microwave heating and pan frying tended to increase when heating after thawing.

**Freezing of cooked, minced meat**

Meat patties (Table 5). With regard to freezing rate, immersion freezing in liquid nitrogen (LN<sub>2</sub>) resulted in higher off-

Table 4—Influence of freezing rate, frozen storage time, method and initial temperature of reheating. Summary of repeated ANOVA to separate interacting variables in Table 3

Source	Factors included in ANOVA	Flavor		Juiciness		Tenderness		Reconstitution loss	
		Difference	P	Difference	P	Difference	P	Difference	P
Freezing rate (F)	FXSXRXI	Off-flavor; adv in- intermediate rates	0.01	N.D.			N.D.	Disadv lowest rate	0.01
Storage time (S)	FXSXRXI	Adv S.S.	Tendency	Adv S.S.	Tendency	Adv L.S.	0.05	N.D.	
	FXSXI (pan frying)	Adv S.S.	0.01	Adv S.S.	0.001	Adv L.S.	Tendency	Adv S.S.	Tendency
	FXS (pan-fried thawed)	Adv S.S.	0.001	Adv S.S.	0.001	Adv L.S.	0.001	Adv S.S.	0.001
	FXS (pan-fried frozen)	Adv S.S.	0.01	Adv S.S.	0.05	Adv L.S.	Tendency	N.D.	
Reheating method (R)	FXSXRXI	Adv pan frying	Tendency	Adv microwaves	Tendency	N.D.		Adv microwaves	0.01
	FXRXI (2 mo)	Adv pan frying	0.01	Adv microwaves	Tendency	N.D.		Adv microwaves	0.001
Initial temp of reheating (I)	FXSXRXI	N.D.		N.D.		Adv frozen	Tendency	Adv thawed	Tendency
	FXRXI (2 mo)	N.D.		N.D.		Adv frozen	0.001	Adv thawed	0.01
	FXSXI (pan frying)	N.D.		N.D.		Adv frozen	Tendency	Adv thawed	Tendency
	FXI (pan-fried 2 mo)	Adv thawed	0.01	N.D.		Adv frozen	0.001	Adv thawed	0.01
	FXI (pan-fried 7 mo)	N.D.		N.D.		N.D.		N.D.	

<sup>a</sup> N.D. = No difference; S.S. = Shorter storage; L.S. = Longer storage

flavor scores than did the intermediate rates, while juiciness tended to increase, and centrifugation loss to decrease, with increasing freezing rate. No difference in yield was noted.

Prolonged frozen storage at  $-20^{\circ}\text{C}$  and  $-30^{\circ}\text{C}$  showed increased surface roughness after reconstitution and decreasing flavor and texture scores, but no

important differences between the two storage temperatures. TBA-values were very low, within the range of experimental error, and showed no differences. They are therefore not listed in Table 5.

Of the reconstitution methods, pan frying gave significantly higher surface roughness and higher flavor, juiciness and texture goodness scores than microwave

heating, while the latter method tended to give higher yield, in agreement with the results for sliced beef.

With regard to initial temperature of reheating, the only difference between reconstitution from the thawed and from the frozen condition was in texture goodness score, where thawing prior to reheating resulted in a higher score.

Table 5—Fried meat patties. Combined influence of freezing rate, storage conditions, method and initial temperature of reconstitution

1. Source of variation, degrees of freedom, mean squares and level of statistical significance

Source of variation	dF	Mean squares								
		Appearance			Off-flavor	Juiciness	Texture		Reconstitution loss	Centrifugation loss
		Surface roughness	Goodness	Flavor			Looseness	Goodness		
Freezing rate (F)	3	0.158	2.28	2.507	2.458*	2.958*	0.521	0.972	0.998	381.39***
Storage conditions (S)	2	5.64***	3.76	11.26***	7.97***	0.849	12.14	26.52***	34.25	0.579
Reheating method (R)	1	2.756*	0.521	12.00***	2.521*	13.02***	5.33	18.75***	19.2	—
Initial temperature (I)	1	1.172	9.188	0.00	1.021	0.188	1.33	4.69*	138.7	—
FXS	6	0.148	0.846	0.721	0.370	0.162	0.537	0.22	1.455	2.99
FXR	3	0.394	0.076	2.347	0.951	0.118	0.389	0.250	2.23	—
FXI	3	0.255	1.465	1.014	0.479	0.285	0.389	0.188	7.70	—
SXR	2	0.318	9.85***	0.109	0.537	0.193	16.32***	1.75	62.1***	—
SXI	2	0.765	11.27***	0.766	0.255	1.73	0.412	0.438	154.3***	—
RXI	1	1.505	12.00***	0.521	0.333	1.33	0.0208	1.021	2.0	—
Error MS		0.415	1.046	1.268	0.703	0.824	0.951	1.023	1.131	0.584
Error dF		167	167	167	167	167	167	167	23	36

2. Mean values, number of observations and S.E. of means<sup>a</sup>

Treatment	Appearance scores					Texture scores		Reconstitution loss, %	Centrifugation loss, %
	Surface roughness	Goodness	Flavor score	Off-flavor score	Juiciness score	Looseness	Goodness		
Freezing rate:									
225 cm/hr	3.7	5.1	5.3	2.0a	3.6a	4.1	4.7	6.3	1.6a
20 cm/hr	3.7	4.9	5.8	1.5b	3.4ab	4.0	4.3	6.8	2.2a
3 cm/hr	3.8	5.4	5.8	1.5b	3.3bc	4.0	4.6	6.2	3.6b
0.1 cm/hr	3.8	4.9	5.6	1.7ab	3.0bc	3.8	4.5	6.5	13.6c
No. of observations	48	48	48	48	48	48	48	12	12
S.E.	0.093	0.148	0.163	0.121	0.131	0.141	0.146	0.307	0.220
Frozen storage:									
2 mo, $-20^{\circ}\text{C}$ , $\text{N}_2$	3.4a	5.4	6.1a	1.3a	3.4	4.5	5.3a	5.0	5.5
7 mo, $-20^{\circ}\text{C}$ , $\text{N}_2$	3.8b	5.0	5.5b	1.7ab	3.4	3.8	4.3b	6.5	5.2
7 mo, $-30^{\circ}\text{C}$ , $\text{N}_2$	4.0b	4.9	5.3b	2.0b	3.2	3.7	4.0b	7.9	5.2
No. of observations	64	64	64	64	64	64	64	16	16
S.E.	0.081		0.141	0.105	0.113		0.126		0.191
Reheating method:									
Pan frying	3.9	5.0	5.9	1.6	3.6	4.1	4.8	6.4	
Microwave	3.6	5.1	5.4	1.8	3.1	3.8	4.2	4.9	
No. of observations	96	96	96	96	96	96	96	24	
S.E.	0.066		0.115	0.086	0.093		0.103		
Initial temp:									
Thawed, $+5^{\circ}\text{C}$	3.7	4.9	5.6	1.6	3.3	4.1	4.7	3.6	
Frozen, $-20^{\circ}\text{C}$	3.8	5.3	5.6	1.7	3.4	3.9	4.4	7.7	
No. of observations	96	96	96	96	96	96	96	24	
S.E.	0.066		0.115	0.086	0.093	0.099	0.103		

<sup>a</sup> Where more than two means are compared, different index letter signifies that values are significantly different at least at  $P < 0.05$ .  
\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$



As seen from Table 5, significant interactions were found in appearance and in yield between several variables, and in texture looseness between storage condition and reconstitution method. Further analysis of variance to separate these effects did not give any conclusive results.

#### Comparison between materials and methods

With increasing freezing rate, yield increased for sliced beef in agreement with Andreotti and Ferlinghi (1967), while meat patties were unexpectedly unaffected, in partial disagreement with early personal experience with patties of similar composition, but where freezing had been preceded by a cooling step (Jakobsson and Bengtsson, 1969). For the patties, water-holding ability increased with freezing rate, which was not the case for sliced beef.

No significant differences in appearance were seen after reconstitution, while increasing freezing rate resulted in increasingly paler surface color in the frozen state.

Liquid nitrogen immersion freezing gave an off-flavor, the reason for which is not apparent, but similar results have been noted also in previous experiments. Possibly, the LN<sub>2</sub> bath may act as a cold trap for odorous compounds from the surrounding working area. LN<sub>2</sub>-spray freezing on the other hand, showed a general tendency to higher flavor scores than did lower freezing rates, in agreement with Andreotti and Ferlinghi (1967).

Tenderness and texture scores were affected but little by freezing rate. Juiciness increased with freezing rate for the patties.

In frozen storage a general decrease in sensory quality with time was found for sliced beef and patties. Storage temperatures of -20 and -30°C differed but little for the raw materials and experimental conditions used in this investigation. Only for sliced beef did water-holding ability and yield decrease, and both yield and sensory quality loss of this material were particularly apparent for pan fried thawed samples. For sliced beef an increase in tenderness was actually seen in contrast with the results for frozen storage of raw, sliced beef, recently reported by the authors (Jakobsson and Bengtsson, 1973). In the experiments no initial control was used for practical reasons. For sliced beef and patties the estimated lower and upper limits of normal commercial storage time were chosen, to find out if prolonged storage would change the relative influence of the other treatment variables, our primary interest not being frozen storage time as such.

Reconstitution by microwave heating resulted in higher yield but lower overall sensory quality than pan frying or heating in a convection oven. This is only in partial agreement with Causey and Fenton (1951) in that they claimed both poorest palatability and yield for frozen ham patties reheated by microwave. A possible reason for the flavor negative may be contaminating volatile substances from constructional or other materials present in the microwave tunnel (plastics etc.), but this has not been investigated. In a previous study, however, (Jakobsson and Bengtsson, 1972) we observed heavy contamination from polypropylene inner laminates in plastic pouches during heat treatment, a common constructional material in microwave tunnels. A probable reason for the above difference in yield is that we used a continuous microwave tunnel with good field distribution and controlled field strength, while stationary ovens usually have poorer field distribution and do not permit good heating control. The higher yield found by us for microwaves is probably a function of lower temperature gradient combined with more rapid heating than in pan frying.

For sliced beef an advantage was seen in tenderness for reheating directly from the frozen state. Otherwise, for both sliced beef and patties an advantage was obtained in yield and sensory quality for reheating from the thawed condition.

Minced meat and sliced beef responded somewhat differently to the variables studied, such as in water-holding ability.

Comparing results for cooked meat with previously reported data for raw meat (Jakobsson and Bengtsson, 1973) a marked difference in behavior was seen with regard to frozen storage effect on tenderness of the sliced beef and with regard to yield when heating with or without previous thawing. The cooked beef became more tender with storage and gave lower yield when heating directly from the frozen state, while the reverse was found for raw beef. The difference in yield may be partly explained by the fact that in one case the protein is already heat denatured prior to freezing, considerably altering both water content and water-holding ability. No obvious explanation can be offered for the change in tenderness with storage. There is a possibility that the decrease in water-holding ability and the directional decrease in juiciness with increasing storage time may have influenced the tenderness sensation.

#### CONCLUSIONS

FOR COOKED MEATS, the variables of raw material, processing, storage and reconstitution are interrelated and inter-

act in a sometimes complex manner, particularly for sliced beef, with storage conditions and manner of reconstitution.

Significant differences in one or more quality aspects were found between freezing rates and methods, frozen storage times, reconstitution methods and between reconstitution from the frozen or the thawed condition.

Sliced beef and minced meat responded somewhat differently to the variables studied in several respects. Compared with earlier results with sliced, raw beef, differences in response to frozen storage and initial reconstitution temperature were the most conspicuous.

The results indicate that moderate to high freezing rates should be used for small samples of cooked meat; that frozen storage should preferably be limited to less than a year; and that reconstitution to eating temperature should be made after previous thawing, preferably by conductive heating. However, the negative effects of immersion freezing in liquid nitrogen and of microwave heating on flavor may quite possibly be due to factors other than the freezing and heating methods as such, in which case they may be possible to overcome by proper choice of equipment and constructional materials. If so, the advantage in yield for microwave heating will increase in practical importance.

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## CONTINUOUS PRODUCTION OF BLUE-TYPE CHEESE FLAVOR BY SUBMERGED FERMENTATION OF *Penicillium roqueforti*

### INTRODUCTION

THE CHARACTERISTIC flavor of Blue-type cheeses is largely attributed to short and medium chain fatty acids (Simonart and Mayaudon, 1956), methyl ketones (Patton, 1950; Dartey and Kinsella, 1971; Anderson and Day, 1966) and secondary alcohols (Jackson and Hussong, 1958; Day and Anderson, 1965). Fatty acids function as precursors of methyl ketones (Gehrig and Knight, 1963; Lawrence, 1966; Dartey and Kinsella, 1973) and secondary alcohols (Jackson and Hussong, 1958; Anderson and Day, 1966), in addition to their contribution to the flavor of Blue cheese per se. The chemistry of methyl ketone formation from fatty acids and various factors involved have been extensively studied. The oxidation of fatty acids by spores of *P. roqueforti* has been studied by Gehrig and Knight (1963), Lawrence (1966) and Dartey and Kinsella (1973). Lawrence and Hawke (1968) and Dwivedi and Kinsella (1974) studied the conversion of fatty acids to methyl ketones by *P. roqueforti* mycelium. Watts and Nelson (1963) and Nelson (1970) have described the exploitation of the capacity of *P. roqueforti* spores to convert fatty acids to methyl ketones. They developed a process for the production of Blue cheese flavor using a spore-rich *P. roqueforti* inoculum. This inoculum is produced by culturing the mold in sterile milk-based medium with a relatively high osmotic pressure produced by the addition of salt and/or sugar. The product is used as a Blue cheese flavorant. We have investigated the adaptability of *P. roqueforti* mycelium for continuous culture and its capacity for the production of a Blue-type cheese flavor by submerged fermentation.

### EXPERIMENTAL

#### Preparation of inoculum

*P. roqueforti* spores were grown in a sterilized (121°C/15 min) culture medium composed of 5% malt extract broth, 2% D-glucose and 0.5% yeast extract (Jackson and Hussong, 1958). Approximately 200 mg spore powder of *P. roqueforti* (Midwest Blue Mold Co., Stillwater, Minn.) was aseptically added to 500 ml

culture medium in a 2-liter flask. The flask was left undisturbed at 25°C for 5–6 days until a thick coat of green spores had grown on the surface of the medium. Small quantities of *P. roqueforti* spores were aseptically scraped from the surface of mold mat and transferred into a sterilized corn-steep liquor (52% total solids, CPC International)/sucrose medium containing 1.5g/100 ml of each. The medium was incubated at room temperature for 4 days with stirring.

The inoculum of *P. roqueforti* mycelium for continuous cultivation of mold was prepared by inoculating 1 liter sterilized corn-steep liquor/sucrose medium (1g/100 ml of each) with the mycelial culture grown as above and incubating it at 25°C with stirring. This was suitable for continuous cultivation after 3 days.

#### Lipolyzed milk fat for carbonyl production

Because the lipase activity of young mycelial culture of *P. roqueforti* is limited, lipolyzed milk fat was used as substrate for continuous flavor production. Heavy cream (32% fat) was diluted with water (1:1). A crude pancreatic lipase preparation (Steapsin, Nutritional Biochem. Corp.) was added to the diluted cream (5g Steapsin/1000 ml). The contents were thoroughly stirred and left at 25°C for 4–5 days. During this period about 80% of triglycerides were hydrolyzed to free-fatty acids and glycerol.

#### Continuous process for flavor production

A flow diagram of the process used for the continuous production of the Blue cheese flavor is shown in Figure 1. All glassware and tub-

ing were autoclaved for 1 hr at 121°C and connected aseptically after cooling. The corn-steep liquor/sucrose medium was adjusted to pH 7.0 with 1.0N NaOH and sterilized at 121°C for 15 min. After cooling, the medium was aseptically transferred to growth medium vessel (#1). 500 ml inoculum was transferred to the culture vessel (#2) and continuous cultivation of *P. roqueforti* was started. The dilution rate was at 0.05 hr<sup>-1</sup> thus providing a doubling time of 20 hr for mold growth. The aeration rate was 1 liter air/min/L medium and temperature was maintained at 25°C. Mold was grown continuously by this process for 3–4 days and collected in a sterile effluent vessel until stable mold growth was achieved. The presence of *P. roqueforti* mycelium was confirmed by microscopic examination. After steady mold growth was achieved, the culture overflow from vessel #2 was connected to aging vessel #3. An average aging period of 24 hr was allowed at this stage. The mycelial culture from vessel #3 was pumped to vessel #4. Lipolyzed milk fat was also continuously pumped into this vessel. The free-fatty acids and buffer concentration in lipolyzed milk fat substrate in vessel #5 was adjusted such that the concentrations of free-fatty acid and phosphate buffer in flavor production vessel #4 was 20 μmoles fatty acids/mg mold mycelium (dry matter basis) and 0.1M respectively. The free-fatty acids present in lipolyzed milk fat were metabolized aerobically by the *P. roqueforti* mycelium into CO<sub>2</sub> and various carbonyl compounds. The average contact time of mold mycelium and free-fatty acids was 24 hr. The fermented liquid product was cooled to 4°C and stored under refrigeration

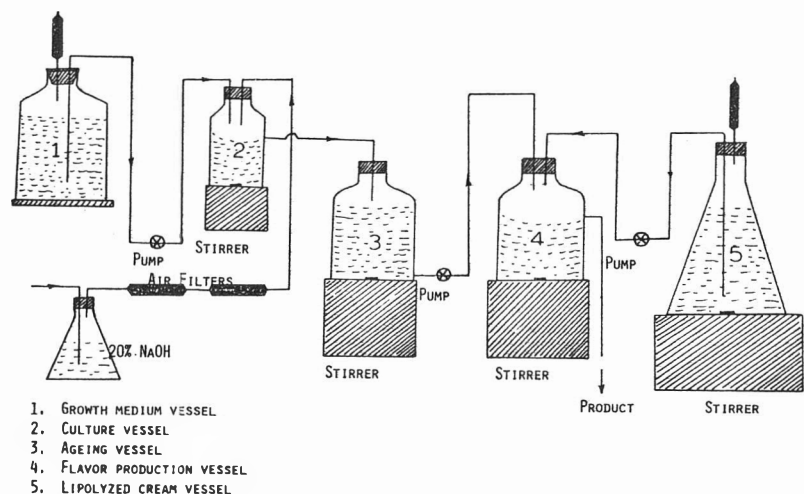


Fig. 1—Flow diagram of continuous production of Blue cheese flavor

<sup>1</sup> Present address: Foster D. Snell, Inc., 3 Hanover Rd., Florham Park, NJ 07932

(4°C) for analysis and evaluation. The continuous fermentation was carried out for 2 wk. No loss in the efficiency of carbonyl production was noticed during this period.

**Free-fatty acid (FFA) determination**

FFA concentration of product was determined by colorimetric method (Anderson and McCarty, 1972). The fatty acid composition was analyzed as butyl esters by gas-liquid chromatography (GLC) using flame ionization detector under the following conditions:

Column: 15% DEGS on G Chrom P 80/100

Column temp: Isothermal 70°C for 10 min then programmed to 210°C/6 C/min

Injection port temp: 325°C.

**Determination of carbonyl compounds**

The 2,4-dinitrophenyl (DNP) hydrazones of carbonyl compounds in the fermented flavor product were prepared by the method of Lawrence (1965). The concentration of total carbonyl DNP hydrazones was determined using the molar extinction coefficient,  $E = 22,500$  (Schwartz and Parks, 1963). Since methyl ketones accounted for more than 90% of total carbonyls in the product, further separation of monocarbonyls by column chromatography was found unnecessary. The total carbonyl DNP hydrazones were concentrated and the individual methyl ketones separated by thin-layer chromatography (Schwartz et al., 1968). A standard mixture of homologous methyl ketone DNP hydrazones ( $C_5$  to  $C_{13}$ ) was spotted on the TLC plate for identification of methyl ketones in the sample. After developing the plates, spots containing methyl ketones ( $C_5$ ,  $C_7$ ,  $C_9$ ,  $C_{11}$ ) were scraped from the plate and the ketones eluted with 5 ml diethyl ether. After evaporating the diethyl ether, DNP hydrazones of methyl ketones were redissolved in n-hexane and their concentration determined from their respective optical density at 340  $\mu$ .

**Sensory evaluation**

Both difference and preference testing was used to evaluate the fermented product, using a good quality Blue cheese as a standard. Samples were prepared by mixing Blue cheese + water (10% of each) or fermented product + cottage cheese (10% of each) in a French onion dip. Samples were thoroughly mixed and stored under refrigeration (4°C) before presenting them to an eight-member panel who were familiar with the flavor of Blue cheese and used it frequently. Panelists were asked to evaluate the flavor of coded Blue cheese flavored onion dip samples on a hedonic scale. For different testing, a triangle test (presenting two similar and one different in a set of three samples) was used and panelists were asked to select the odd sample.

**RESULTS & DISCUSSION**

THE FERMENTED PRODUCT obtained by two stage continuous fermentation was a light tan liquid resembling milk. The average composition of the product (8 analyses) was: 4.2% total solids, 0.3% protein, 1.2% lipids, 0.6% free-fatty acids and 0.04% total carbonyls. Significant variation in the yield of total carbonyls was observed from batch to batch depending on the yield of mold myce-

**Table 1—Sensory evaluation of fermented Blue-type cheese flavor product**

Product	Flavor <sup>a</sup>	
	Score	Description
French onion chip-dip + fermented product	9.5	Like moderately-like extremely
French onion chip-dip + Blue cheese	9.0	Like moderately

<sup>a</sup> Triangular comparison: No significant difference at 5% and 1% level.

**Table 2—Concentrations of methyl ketones in fermented Blue-type cheese flavor and Blue cheese**

Methyl ketone	Concentration mg/100g	
	Blue cheese <sup>a</sup>	Fermented product
2-Pentanone	1.52	3.2
2-Heptanone	3.48	8.3
2-Nonanone	3.31	15.0
2-Undecanone	0.85	2.2

<sup>a</sup> Anderson and Day (1966)

**Table 3—Concentrations of free-fatty acids in fermented Blue-type cheese flavor product and Blue cheese**

Fatty acid	Concentration mg/100g	
	Blue cheese <sup>a</sup>	Fermented product
4:0	117	—
6:0	79	Tr
8:0	75	7.2
10:0	134	40.5
12:0	143	27.5
14:0	496	89.5
16:0	1088	165.5

<sup>a</sup> Anderson and Day (1966)

lium. True steady state for mold growth was not achieved because the mycelium had the tendency to accumulate near the orifice of culture outlet vessel #2 and around the probes entering the fermentation vessel. The fermentation vessel was so designed that all the probes (for aeration, medium input, etc.) were above the surface of liquid. To circumvent the problem of mycelial accumulation near the orifice of culture outlet a device similar to one suggested by Brunner and Röhr (1972) is being tested.

The sensory evaluation data are presented in Table 1. No significant difference was observed in the Blue cheese character of chip-dip irrespective of flavor source, i.e., fermented product or Blue cheese.

The average concentrations of  $C_5$ ,  $C_7$ ,  $C_9$  and  $C_{11}$  methyl ketones in the fermented produce and Blue cheese are given in Table 2. Acetone,  $C_{13}$  and  $C_{15}$

methyl ketones were also detected in the fermented product. However, the concentration of these ketones was very small and could not be measured accurately. The concentrations of  $C_5$ ,  $C_7$  and  $C_{11}$  methyl ketones in fermented product were twice, and  $C_9$  methyl ketone four times, that of Blue cheese. The flavor panel found the intensity of the Blue cheese flavor in the fermented product similar to Blue cheese in spite of the higher methyl ketone concentration in the former (Table 1, 2). Differences in the concentration of protein, amino acid, peptides, lipid, carbohydrates, etc. and the degree of interaction of methyl ketones with some of these components may account for variable flavor intensity of methyl ketones in the fermented product and Blue cheese.

A comparison of the concentration of individual fatty acids in the fermented product and Blue cheese is given in Table

3. The fermented product had low concentrations of free-fatty acids compared to Blue cheese. The short chain fatty acids impart a "peppery taste" to Blue cheese. The short chain fatty acids impart a "peppery taste" to Blue cheese (Currie, 1914), which is considered desirable. The apparent difference in the concentrations of free-fatty acids in the fermented product and Blue cheese did not show up in the flavor panel study using chip-dip as the base (Table 1). It is quite possible that the lack of C<sub>6</sub>, C<sub>8</sub> and C<sub>10</sub> free-fatty acids in the fermented product may become apparent when added to some other products.

The continuous process for the production of Blue cheese-type flavor by submerged fermentation is promising. This process makes it possible to change the relative concentrations of major methyl ketones by changing the free-fatty acid concentration in the fermentation medium (Dwivedi and Kinsella, 1974), and may be helpful in obtaining a desirable flavor balance to suit an individual food product.

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## A NUMERICAL METHOD OF SIMULATING THE AXISYMMETRICAL FREEZING OF FOOD SYSTEMS

### INTRODUCTION

FREEZING OF FOOD is a moving boundary problem and has its mathematical analogs in thawing and oxidation of solids including combustion. This moving boundary problem is important in preservation of food, blood, tissues and microbes; casting, prilling, extrusion of plastics and metals; geographical phenomenon of freezing waterlogged grounds; and others.

Most of the previous study of simulating a freezing phenomenon involves a pure liquid which freezes at a constant temperature. Several methods such as numerical (Longwell, 1958; Morrison, 1970; Tao, 1967), analog (Baxter, 1962) and analytical (Carslaw and Jaeger, 1959) are available. Since many processed systems are mixtures, freezing temperatures of such a system become lower as more liquid freezes. Also, the spatial and temporal distribution of temperature often affects properties of the processed product. Numerical methods for freezing a semi-infinite slab with simplified assumptions (Rohatgi and Adams, 1967; Tien and Geiger, 1967) have been proposed and analyzed. This study describes a numerical method with more rigor to simulate the temperature history of freezing a macroscopically homogeneous, axisymmetric system in which no macroscopic transport of mass occurs.

### THEORY

THE MAIN difficulty of simulating a freezing mixture is that the solids content changes continuously for temperatures below the freezing point. If the following assumptions are made, the transport behavior of freezing a slab, a cylinder, or a sphere can be conveniently described by using enthalpy as the independent variable shown by equations (1) to (4).

#### Assumptions

1. No change of initially uniform macroscopic composition except phase transition;
2. Constant density or no hydrodynamic velocity which implies negligible volumetric change of the system even though local voidage may vary;
3. Smooth interphase boundary; and
4. No supercooling so that known equilibrium physical properties, such as enthalpy  $H(T)$ , liquid fraction frozen  $x(T)$  and thermal conductivity  $k(T)$ , can be used.

Table 1—Comparison of time to freeze a pure liquid slab initially at  $T^* = 1.50$

Interface position in $(r_i/40)$ units from $r_i$	$t^*$		$T^*$ at Center
	this method	Neumann <sup>a</sup>	
0.5	0.00002	0.00005	1.5000
4.5	0.00385	0.00380	1.5000
8.5	0.01375	0.01356	1.5000
12.5	0.02965	0.02933	1.4985
16.5	0.05150	0.05110	1.4727
20.4	0.07922	0.07888	1.3854

<sup>a</sup> Carslaw and Jaeger (1959)

#### Describing equations

$$\rho \frac{\partial H}{\partial t} = k \left( \frac{\partial^2 T}{\partial r^2} + \frac{a}{r} \frac{\partial T}{\partial r} \right) + \frac{\partial k}{\partial r} \frac{\partial T}{\partial r} \quad (1)$$

$$T = T_i \text{ at } t = 0 \quad (2)$$

At  $r = r_i$ ,

$$k \frac{\partial T}{\partial r} = -U (T - T_c) \text{ and } T = T_c \text{ for } U = \infty \quad (3)$$

$$\frac{\partial T}{\partial r} = 0 \text{ at } r = 0 \quad (4)$$

In order to minimize the number of independent variables and to generalize analogous systems, these equations may be rearranged to (1A) to (4A) by using dimensionless terms similar to previous work on pure liquid (Tao, 1967):

$$\frac{\partial H^*}{\partial t^*} = k^* \left( \frac{\partial^2 T^*}{\partial r^{*2}} \right) + \left( \frac{\partial k^*}{\partial r^*} \right) \left( \frac{\partial T^*}{\partial r^*} \right) \quad (1A)$$

$$T^* = T_i^* \text{ at } t^* = 0 \quad (2A)$$

$$\left. \begin{aligned} \frac{\partial T^*}{\partial r^*} &= -\frac{T^*}{\beta} \text{ for } \beta \neq 0 \\ T^* &= 0 \text{ for } \beta = 0 \end{aligned} \right\} \text{ at } r^* = 1 \quad (3A)$$

$$\frac{\partial H^*}{\partial t^*} = k^*(1+a) \frac{\partial^2 T^*}{\partial r^{*2}} \text{ at } r^* = 1 \quad (4A)$$

Equation (4A) is obtained by substituting equation (4) into equation (1) at  $r = 0$ . For the term  $(\partial T/\partial r)/r$  in equation (1), L'Hopital rule is used to obtain  $\partial^2 T/\partial r^2$  as its replacement. These equations can be further transformed to difference equations by using the forward difference for time derivative and the central differences for space derivative to result in equations (1B) to (4B). The space increments are numbered progressively from  $r_i$  (grid point 1) to the center (grid point  $M+1$ ).

$$\begin{aligned} H_{m,n+1}^* &= H_{m,n}^* + \left( \frac{\Delta t^*}{\Delta r^{*2}} \right) \left[ k_{m,n}^* \left( \left( 1 + \frac{a}{2(M-m+1)} \right) T_{m-1,n}^* - 2T_{m,n}^* \right. \right. \\ &\quad \left. \left. + \left( 1 - \frac{a}{2(M-m+1)} \right) T_{m+1,n}^* \right) + 1/4(k_{m-1,n}^* - k_{m+1,n}^*) \right. \\ &\quad \left. (T_{m-1,n}^* - T_{m+1,n}^*) \right] \end{aligned} \quad (1B)$$

$$T^* = T_i^* \text{ at } t^* = 0 \quad (2B)$$

$$T_{1,n}^* = T_{2,n}^* / (1 + \Delta r^*/\beta) \text{ for } \beta \neq 0 \text{ and } T_{1,n}^* = 0 \text{ for } \beta = 0 \quad (3B)$$

$$H_{M+1,n+1}^* = H_{M+1,n}^* + 2 \left( \frac{\Delta t^*}{\Delta r^{*2}} \right) k_{M+1,n}^* (1+a) (T_{M,n}^* - T_{M+1,n}^*) \quad (4B)$$

Besides these equations, physical properties of the system in terms of equation (5), (6) and (7) must also be available.

To start, all grid points shall be set at the given initial temperature and its corresponding enthalpy.  $k^*$  at each point is then calculated by equation (5).

$$k^* = k^*(T^*, x) \tag{5}$$

$$H^* = H^*(T^*) \tag{6}$$

$$x = x(T^*) \text{ for } T^* < 1.0 \text{ or } H^* < 1.0 \tag{7}$$

This allows one to compute enthalpy values of the next time increment for grid point 2 to M by using equation (1B). The center point is to be calculated by equation (4B) and the grid point 1 by using equation (3B). From the calculated enthalpy values of the grid points, respective temperatures are evaluated by equation (6). This cycle of calculation is thus repeated to obtain the temporal and spatial temperature profiles of a system during the freezing operation. When  $T^*$  or  $H^*$  becomes less than 1.0, equation (7) is used to calculate the fraction of water frozen so that  $k^*$  of the partially frozen mixture can be obtained from equation (5).

To test this numerical method, calculations are performed for a pure liquid. The results agree well with Neumann's (Carslaw and Jaeger, 1959) analytical solution. However, it must be cautioned that an agreement with an asymptotic solution is only a necessary but not sufficient condition.

**EXPERIMENTAL**

FOOD SYSTEMS are generally macroscopically homogeneous, as the cell membranes prevented substantial macroscopic movement of constituents. Therefore, they appear suitable for the present simulation study. Also, a recent review (Holdsworth, 1970) indicated precise modeling is not available due to the complexity of the phenomenon. Furthermore, temperature transients in a freezing operation effect deactivation of enzymes, movement of water from intracellular to intercellular spaces and affect ice crystal size distribution. These directly influence the physical, chemical and nutritional properties of frozen foods. Thus, successful simulation may be used for improving the freezing operation of foods. A 1.5-in. thick beef slab was chosen for this study.

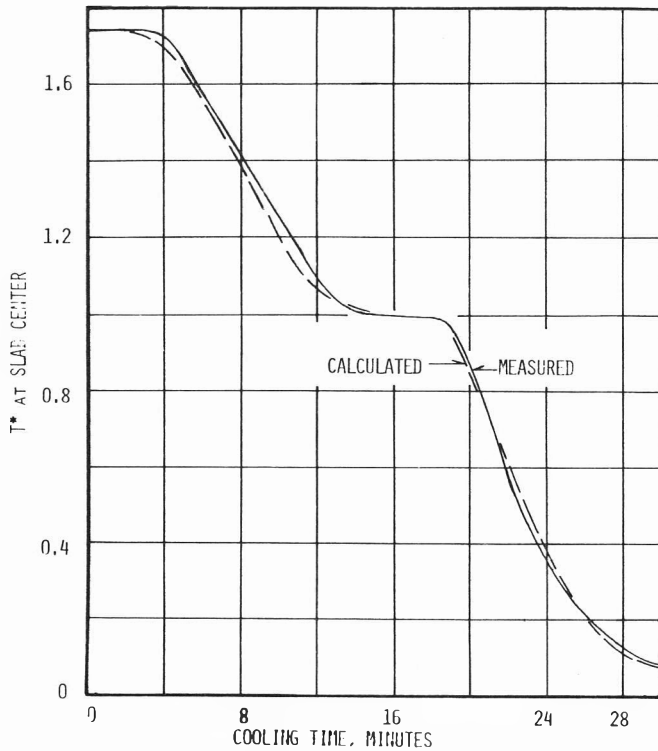


Fig. 1—A comparison of measured and calculated temperature progressions.

A mixture of ethylene glycol and water was used as the cooling bath. It was agitated vigorously with a stirrer controlled by a powerstat. A styrofoam block with a 1.5 by 1.5 in. cavity was used as a sample holder for two pieces of food samples each of 0.75 in. thickness. Sandwiched between the two food samples is a 0.002 in. bead of a copper-constantan thermocouple (Omega Engineering Co.) the output of which is printed out on a Honeywell Elektronik-19 strip-chart recorder. The food sample and the block were bolted together with two 0.0625 in. thick copper plates which had an attached thermocouple for measuring the surface temperature to be maintained at a constant level in order to simplify the computation. Either this thermocouple or the other one immersed in the liquid bath can be selected for reading out from a digital multimeter (Data Technology model 370). Detail constructions are shown elsewhere (Joshi, 1972).

Two pieces of selected beef steak (26.73% solids) were cut to fit snugly into the cavity. Muscle fibers were parallel to the direction of heat flow. A thin layer of silicone grease was then applied to the styrofoam block surface in contact with the copper plates which were bolted on later. This was found necessary to prevent the bath liquid from seeping into the food surface and sample cavity.

The sample was at first equilibrated in room temperature  $T_i = 77.5^\circ\text{F}$  while the bath was adjusted to  $-39.0^\circ\text{F}$  as  $T_c$ . Then, the sample was lowered to the bath and a mark was made on the strip-chart for timing. A continual adjustment of the bath temperature was made especially in the beginning to maintain the copper plates at  $-39.0 \pm 1^\circ\text{F}$ . This was accomplished by manually controlling the expansion valve of the refrigeration unit. As the thermal conductivity of copper plate is about 300 times larger than that of beef,  $\beta = 0$  can be assumed to simplify the computation process. The run was concluded after about 30 minutes of cooling and the temperature curve at the center was replotted in terms of dimensionless temperature and real time on Figure 1.

**CALCULATIONS & DISCUSSION**

FOR BEEF, some reported properties are available (Long, 1955; Dickerson, 1968). They are approximated (Joshi, 1972) by equation (5A), (6A) and (7A).

$$k^* = (1 + x)/(1 - 0.5x) \tag{5A}$$

$$H^* = 1 + 0.84 (T^* - 1) (T_f - T_c)/H_f \text{ for } T^* \geq 1.0$$

$$\frac{T_c + 50}{T_f + 50} + \frac{T_f - T_c}{T_f + 50} T^* = \frac{1 - \exp(-5.75 H^*)}{1 - \exp(-5.75)} \text{ for } T^* \leq 1.0 \tag{6A}$$

$$x = 0 \text{ for } T^* > 1.0$$

$$x = 0.923 \left[ 1 - \frac{0.025}{1 - 0.975 \left( \frac{T_c + 50}{T_f + 50} + \frac{T_f - T_c}{T_f + 50} T^* \right)} \right] \tag{7A}$$

for  $T^* < 1.0$

The physical properties for computing the dimensionless parameters are from Tressler et al. (1968):

$$\rho = 72.4 \text{ lb/cu ft}$$

$$k_f = 0.26 \text{ Btu/(hr) (ft) } (^\circ\text{F})$$

$$T_f = 30.4^\circ\text{F}$$

$$H_f = 133.9 \text{ Btu/lb with } 50^\circ\text{F as the datum temperature.}$$

Calculation in an IBM 360 computer by using  $M = 40$  and  $\Delta t^*/\Delta r^*{}^2 = 0.027$  results in a freezing curve simulating fairly well the experimental results in Figure 1. Approximately 5 min were used.

In simulating the behavior of a system, there are three general approaches. Prior to availability of electronic computers, empirical correlations of measured data were used and are still in use for design purposes. Later, to assure that simulation results follow the laws of nature and meanwhile to avoid tedious or difficult solution of rigorous equations, the semi-theoretical approach has often been used such as to divide a

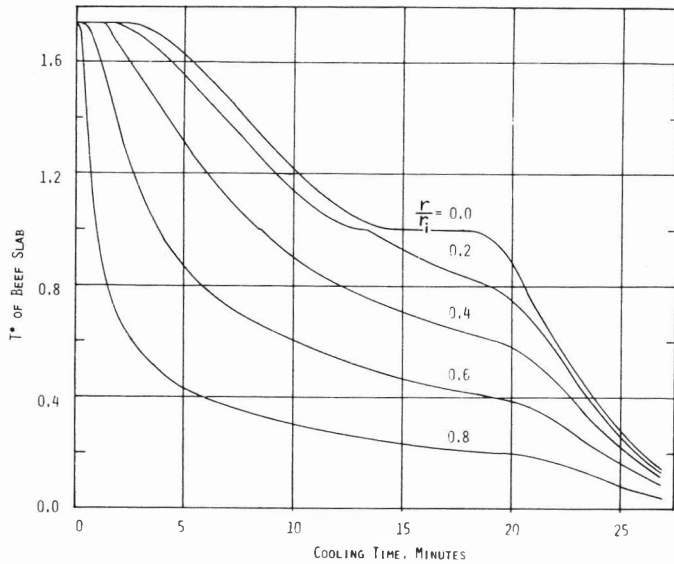


Fig. 2—Calculated temperature progressions at various locations.

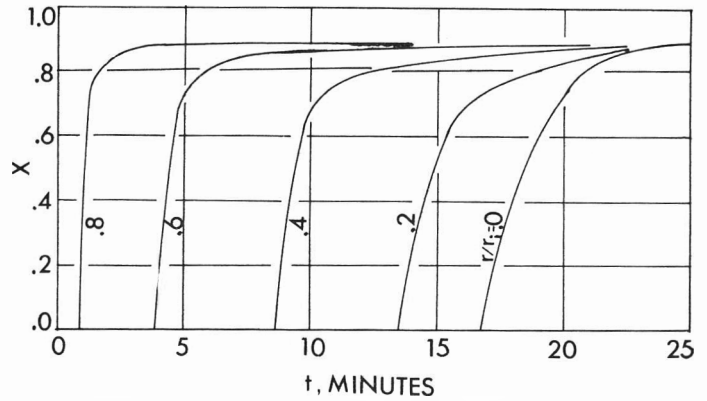


Fig. 4—Calculated fractions of water frozen versus time at several space locations.

continuous region into separate regions for inserting easier solutions and to insert assumptions for facilitating the computation. Since electronic computers became accessible, solution of rigorous equations is preferred so that potential errors derived from empirical assumptions may be eliminated and less experimentation is needed to test the computation results. If the describing equations are completely rigorous, often the calculation results can be more reliable than the measured ones provided that truncation and round-off errors are small.

The purpose of this study was to formulate a theoretically sound method of projecting temperature transient in freezing a mixture. The freezing curve is the measured temperature versus time at the slab center. Good agreement between the meas-

ured and the calculated curves confirms the feasibility of predicting the temperature distributions in a mixture such as a food system provided that physical data  $H(T)$ ,  $k(T)$  and  $x(T)$  of that system are available.

Measurement of temperatures simultaneously at various locations in a freezing mixture is often difficult since the insertion of many probes would affect the structure and cause distortion of the time-temperature profiles. An agreement between the measured and the calculated temperature progression at the slab center not only indirectly substantiates the numerical method but also provides the benefit of retrieval of temperature distribution at various time intervals as well as temperature progressions at other locations. These are shown in Figures 2 and 3. Figure 4 is the frozen water distribution calculated from Figure 2 with equation (7A). Direct and continuous measurement of frozen water in a food system is difficult.

Different numerical methods can be derived from the same set of differential equations. Conditions of convergence of nonlinear differential equations have not been well established and stability of difference equations depends on the number of iterations and the size of increments. For the present case, instability may occur near the interphase region and can be avoided by reducing the time increment size. Convergence of computation results is detected by reducing successively the increment size. Reduction of time increment size to provide stability was incorporated automatically in the computer program.

This proposed numerical method requires a small  $\Delta t^*/\Delta r^{*2}$  value for stability of a solution. This in turn increases the computation time. The longer real freezing time is to be simulated, the smaller this ratio needs to be for obtaining a stable solution. Truncation error may be estimated by variation of grid size (Lapidus, 1962; Tao, 1967) from  $M = 10$  to 60.  $M = 40$  is the optimum size used and the estimated truncation error is less than 0.75% for the present system.

CONCLUSION

A REALISTIC and theoretically sound numerical method is proposed which was found to simulate satisfactorily the freez-

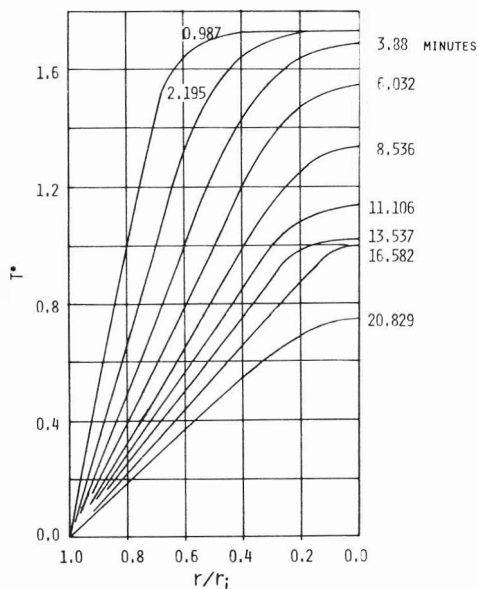


Fig. 3—Calculated temperature distribution.

ing temperature versus time curve at the center of a beef slab. The method requires known physical properties of the freezing food including density, thermal conductivity, enthalpy and fraction of water frozen.

### LIST OF SYMBOLS

a	shape factors = 0, 1, 2 for slab, cylinder and sphere respectively
H	enthalpy, Btu/lb
$H_f$	enthalpy at freezing temperature
$H^*$	dimensionless enthalpy, $H/H_f$
k	thermal conductivity, Btu/(hr) (ft) ( $^{\circ}$ F)
$k_f$	thermal conductivity at $T \geq T_f$
$k^*$	dimensionless conductivity, $k/k_f$
m	space increments number
M	total number of space increments
n	time increments number
r	space position from center of specimen
$r_i$	distance from center to outer surface of specimen
$r^*$	dimensional distance, $r/r_i$
t	time
$t^*$	dimensionless time, $tk_f(T_f - T_c)/\rho H_f r_i^2$
T	temperature, $^{\circ}$ F
$T_c$	temperature of cooling bath
$T_f$	freezing temperature
$T_i$	initial temperature of specimen
$T^*$	dimensionless temperature, $(T - T_c)/(T_f - T_c)$
U	heat transfer coefficient at the specimen surface

x	weight fraction of water frozen
$\beta$	dimensionless term, $k/U r_i$
$\rho$	density, lb/ft <sup>3</sup>

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## ANALYTICAL PREDICTION OF DRYING PERFORMANCE IN NONCONVENTIONAL SHAPES

### INTRODUCTION

THE DIFFUSIONAL FLOW of water is an important part of many food drying processes. In general, several mechanisms are expected when considering a drying process. They are sometimes divided into two broad all-including categories: one in which drying occurs as if the system were pure water being evaporated and one with internal control. The first type may or may not occur, but the second type is always present (Van Arsdel and Copley, 1963). After the pioneering work of Buckingham (1907), the early high moisture stage of drying has been regarded by many authors as surface forces controlled (Luikov, 1948, 1950; Krischer, 1938, 1940, 1942, 1956). According to Krischer (1963) the late phases are controlled by series, parallel or series-parallel combinations of capillary and diffusional mechanisms. Luikov and Mykhaylov (1961) and Luikov (1965, 1967) redefines the moisture transfer driving force on the basis of the theory of thermodynamics of irreversible processes. He allows for a thermogradient effect and defines a "moisture diffusivity" parameter which in essence is an overall coefficient which includes the diffusional effect. But many practical cases in food drying and other drying processes (Ohtani, 1972) can be explained in terms of purely diffusional phenomena, which take place at the cell and at the macrostructure (capillaries, pores) of the foodstuff.

Depending on the particular problem the diffusional flow will be vapor or liquid flow or both; usually one of them controlling (McCready and McCabe, 1933; Krischer, 1938, 1942, 1956; Luikov, 1948; Jason, 1958). The moisture migration equation for capillary flow, when the gradient of chemical potential of the moisture is considered, turns out to be formally similar to Fick's second law with concentration-dependent diffusion coefficient (Harmathy, 1969). A thorough review of the diffusional transfer of water during the drying of solid foodstuffs was made by Van Arsdel et al. (1963). Gurr et al. (1952) have suggested the use of an evaporation-condensation approach. It has been used primarily for textile materials (Henry, 1939; Cassie et al., 1940; Walker, 1961).

When dealing with diffusion-controlled drying processes, it is important to solve the diffusion equation and integrate it over the volume to yield an equation to predict weight loss as a function of time and geometry. This is difficult to do with nonconventional shapes and usually it is done numerically. In the present paper an analytical solution is given and it is applied to the drying of alternate apple cuts.

#### Analytical solution for conventional shapes

If the shapes analyzed are natural to one of the common coordinate systems for which the governing partial differential equation can be solved by the method of separation of variables, the mathematical approach is fairly simple. The unsteady diffusion equation is stated:

$$\partial C / \partial t = \text{div} (D \text{ grad } C) \quad (1)$$

and it is solved analytically, taking into account the initial and

boundary conditions. Several examples have been worked out by Carslaw and Jaeger (1947), Crank (1956), and other authors. For instance, if the system accepts cartesian coordinates

$$C = C(x, y, z, t) \quad (2)$$

and one assumes:

$$C = X(x) \cdot Y(y) \cdot Z(z) \cdot T(t) \quad (3)$$

the solution is straightforward.

#### Solution for nonconventional shapes

If the shapes considered do not have natural coordinate systems on the  $x, y$  plane, the usual approach is a numerical technique. An approximate analytical solution is possible using conformal mapping (Laura and Chi, 1965). Briefly, the dependence on the  $x, y$  plane can be written

$$\nabla_1^2 U(x, y) + \gamma_p^2 U(x, y) = 0 \quad (4)$$

The nonconventional shape on the  $w = x + iy$  plane is transformed into a unit circle in another plane  $\xi = r \cdot e^{i\theta}$ . Equation (4) on this plane becomes

$$4 \frac{\partial^2 U}{\partial \xi \partial \bar{\xi}} + \gamma_p^2 f'(|\xi|)^2 \cdot U = 0 \quad (5)$$

The typical initial and boundary conditions for drying are:

$$C(x, y, z, 0) = C_0 \quad (6)$$

$$C[F(x, y, z) = 0, t] = 0 \quad (7)$$

where  $C$  can be regarded as total or relative concentration and  $F(x, y, z) = 0$  defines the surface of the body. For these conditions, the solution can be expressed in terms of cylindrical harmonics and the  $\theta$  dependent terms of series can be disregarded (Laura and Chi, 1965). Using one of the weighted residue techniques a truncated series is assumed:

$$U = \sum_{p=0}^P A_{0p} J_0(\alpha_{0p} r) \quad (8)$$

and the "colocation along arcs" criterion is used to minimize the error or residual function (Laura and Chi, 1965).

The  $\gamma_p$ 's are calculated from the determinantal equation which results from the homogeneous system of linear equations in the  $A_{0p}$ 's. This system is obtained by equating the total error along  $P$  arcs to zero. The boundary condition for  $r = 1$  shows that the  $\alpha_{0p}$ 's are the roots of the Bessel function of the first kind and zero order. Thus,

$$C = \sum_{m=0}^M \sum_{p=0}^P A_{0mp} J_0(\alpha_{0p} r) \cos \frac{(2m+1)\pi z}{2L} \cdot \exp \left\{ -Dt \left[ \frac{(2p+1)^2 \pi^2}{4L^2} + \frac{\gamma_p^2}{\rho^2} \right] \right\} \quad (9)$$

The values of  $A_{0mp}$  are obtained by multiplying the initial condition as it results from equation (9) by  $r J_0(\alpha_{op} r)$ ,

$$C_0 \cdot r J_0(\alpha_{op} r) = r J_0(\alpha_{op} r) \tag{10}$$

$$\sum_{m=0}^M \sum_{p=0}^P A_{0mp} J_0(\alpha_{op} r) \cos \frac{(2m+1)z\pi}{2L}$$

and integrating from  $r = 0$  to  $r = 1$

$$\frac{C_0}{\alpha_{op}} J_1(\alpha_{op}) = \sum_{m=0}^M A_{0mp} \cos \frac{(2m+1)\pi z}{2L} \cdot \frac{1}{2} J_1^2(\alpha_{op}) \tag{11}$$

Multiplying both sides of equation (11) by  $\cos(2m+1)\pi z/2L$  and integrating from  $z = -L$  to  $z = +L$ , it yields

$$A_{0mp} = \frac{8 C_0}{(2m+1)} \frac{(-1)^m}{\alpha_{op} J_1(\alpha_{op})} \tag{12}$$

The concentration distribution results from equation (9) and (12)

$$\frac{C}{C_0} = \frac{8}{\pi} \sum_{m=0}^M \sum_{p=0}^P \frac{(-1)^m}{(2m+1)} \frac{J_0(\alpha_{op} r)}{\alpha_{op} J_1(\alpha_{op})} \cos \frac{(2m+1)\pi z}{2L} e^{-Dt \left( \frac{(2p+1)^2 \pi^2}{4L^2} + \frac{\gamma_p^2}{r^2} \right)} \tag{13}$$

When drying experiments are considered, the total amount of moisture diffusing from the solid body is more useful than the concentration distribution, due to the experimental difficulty of obtaining data for the latter. Equation (13) is integrated for different shapes, to obtain the  $M_t/M_0$  ratio.

For the cardioidal cross section (Fig. 1)

$$M_t = \int_0^1 \int_0^1 \int_{-L}^L \sum_{m=0}^M \sum_{p=0}^P \frac{8 C_0}{\pi \alpha_{op} J_1(\alpha_{op})} \frac{(-1)^m J_0(\alpha_{op} r)}{(2m+1)} \cos b_m z e^{-F_p^h Dt} \cdot l_c^2 (1 + 2r \cos \theta + r^2) \cdot r dr d\theta dz \tag{14}$$

which yields, equation (16), taking into account equation (15)

$$\int_0^1 r^{(2k+1)} J_0(\alpha_{op} r) dr = \left\{ \sum_{j=0}^k (-1)^j 2^{2j} \left[ \frac{k!}{(k-j)!} \right]^2 \alpha_{op}^{2(k-j)+1} \right\} \frac{J_1(\alpha_{op})}{\alpha_{op}^{(2k+2)}} \tag{15}$$

Where  $k = 0, 1, 2 \dots$

$$\frac{M_t}{M_0} = \frac{4}{3} \frac{32}{\pi^2} \sum_{m=0}^M \sum_{p=0}^P \frac{e^{-F_p^h Dt}}{(2m+1)^2} \frac{1}{\alpha_{op}^2} \left( 1 - \frac{2}{\alpha_{op}^2} \right) \tag{16}$$

For the circle

$$\frac{M_t}{M_0} = \frac{32}{\pi^2} \sum_{m=0}^M \sum_{p=0}^P \frac{e^{-F_p^h Dt}}{(2m+1)^2} \frac{1}{\alpha_{op}^2} \tag{17}$$

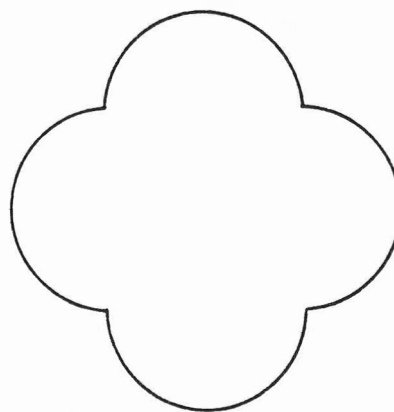


Fig. 1—Cardioidal cross section.

For the hexagon

$$M_t = \int_0^1 \int_0^1 \int_{-L}^L \sum_{m=0}^M \sum_{p=0}^P \frac{8 C_0}{(2m+1)} \frac{(-1)^m}{\alpha_{op} J_1(\alpha_{op})} \cos b_m z e^{-F_p^h Dt} J_0(\alpha_{op} r) l_h \cdot Q r dr d\theta dz \tag{18}$$

where

$$Q = A + 2B \Sigma_3 + 20 \Sigma_6 + 2D \Sigma_9 + 2E \Sigma_{12} + 2F \Sigma_{15} \tag{19}$$

$$A = a^2 + b^2 r^{12} + c^2 r^{24} + d^2 r^{36} + e^2 r^{48} + f^2 r^{60} \tag{20}$$

$$B = abr^6 + bcr^{18} + cdr^{30} + der^{42} + efr^{54} \tag{21}$$

$$C = acr^{12} + bdr^{24} + cdr^{36} + dfr^{48} \tag{22}$$

$$D = adr^{18} + bcr^{30} + cfr^{42} \tag{23}$$

$$E = aer^{24} + bfr^{36} \tag{24}$$

$$F = afr^{30} \tag{25}$$

$$\Sigma_\beta = \sum_{j=0}^{\beta} \frac{(-1)^j (2\beta)!}{(2j)! (2\beta-2j)!} (\cos \theta)^{2\beta-2j} \sin^2 \theta^j \tag{26}$$

The coefficients  $a, b, \dots, f$  result from the polynomial mapping function  $w = f(\xi)$  (Kantorovich and Krilov, 1964; Laura and Chi, 1965; Casarella et al., 1967). They are:  $a = 1.0376$ ;  $b = -0.3338$ ;  $c = 0.2262$ ,  $d = -0.1900$ ;  $e = 0.2275$ ; and  $f = -0.1457$ .

When the integration over  $\theta$  is carried on, all but the A term vanish, since it can be easily shown that

$$\Sigma_\beta = \cos 2\beta\theta \tag{27}$$

Finally,

$$\frac{M_t}{M_0} = 0.9060 \frac{32}{\pi^2} \sum_{m=0}^M \sum_{p=0}^P \frac{e^{-F_p^h Dt}}{(2m+1)^2} \frac{1}{\alpha_{op}^2} \cdot S_p^h \tag{28}$$

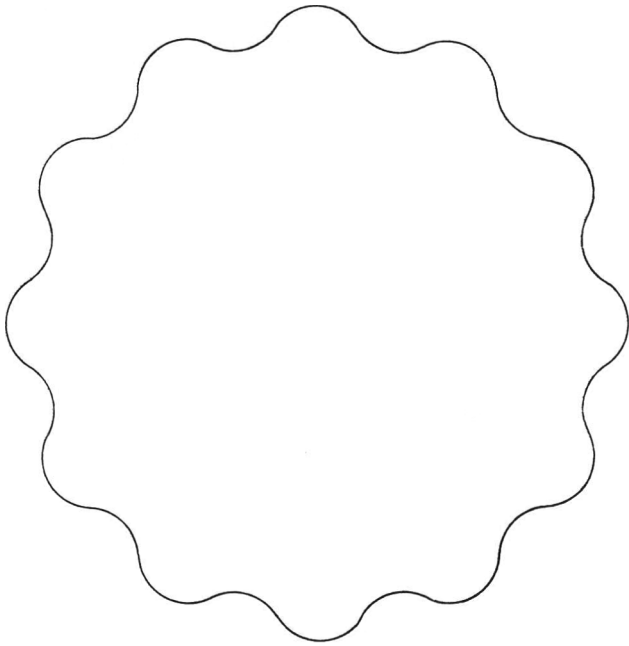


Fig. 2-Corrugated cross section (loop 1/8).

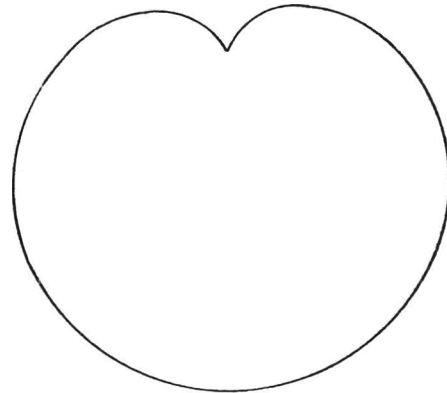


Fig. 3-Epitrochoidal cross section

where

$$S_p^h = a^2 + b^2 \sigma_6 + c^2 \sigma_{12} + d^2 \sigma_{18} + e^2 \sigma_{24} + f^2 \sigma_{30} \quad (29)$$

Where  $\sigma_k$  is given by the following functional relation:

$$\sigma_k = \sum_{j=0}^k (-1)^j 2^{2j} \left[ \frac{k!}{(k-j)!} \right]^2 \alpha_{op}^{-2j} \quad (30)$$

By an analogous procedure, the ratio of diffusing substance for a square cross section will be:

$$\frac{M_t}{M_o} = 0.9651 \frac{32}{\pi^2} \sum_{m=0}^M \sum_{p=0}^P \frac{e^{-F_p^s Dt}}{(2m+1)^2} \frac{1}{\alpha_{op}^2} S_p^s \quad (31)$$

where

$$S_p^s = 1.080^2 + 0.5425^2 \sigma_4 + 0.0450^2 \sigma_8 + 0.0026^2 \sigma_{12} \quad (32)$$

If a corrugated cross section is considered, a similar derivation will yield (Fig. 2):

$$\frac{M_t}{M_o} = 0.9524 \frac{32}{\pi^2} \sum_{m=0}^M \sum_{p=0}^P \frac{e^{-F_p^s Dt}}{(2m+1)^2} \frac{1}{\alpha_{op}^2} \left[ 1 + \left(\frac{13}{16}\right)^2 \sigma_{12} \right] \quad (33)$$

In the case of an epitrochoid, one obtains (Fig. 3):

$$\frac{M_t}{M_o} = 0.9119 \frac{32}{\pi^2} \sum_{m=0}^M \sum_{p=0}^P \frac{e^{-F_p^e Dt}}{(2m+1)^2} \frac{1}{\alpha_{op}^2} \left[ 1 + \frac{25}{36} \sigma_4 \right] \quad (34)$$

Equations (16), (17), (28), (31), (33) and (34) are useful to calculate  $M_t/M_o$  as a function of time and geometrical parameters, for cardioid, circular, hexagonal, square, corrugated and epitrochoidal shapes.

**Performance comparison between different shapes**

When drying single pieces of a foodstuff, it can be useful to compare the relative performance of each. This requires setting a base of comparison. In this case the basis is an equal weight-equal cross sectional area (or equal weight-equal thickness). Equation (35) shows that either one is the same:

$$V = A_1 \times 2 L_1 = A_2 \times 2 L_2 \quad (35)$$

Table 1 gives the area for different shapes.

Table 1-Cross sectional area for different shapes

cardioid	circle	corrugated	square	epitrochoid	hexagon
$\frac{3}{2} \pi l_c^2$	$\pi l^2$	$\frac{129}{128} \pi l_r^2$	$4 l_s^2$	$\frac{41 \pi}{36} l_e^2$	$\frac{3\sqrt{3}}{2} l_h^2$

**Application to apple drying**

The drying of apples has been studied by several authors, with different aims. Ede and Hales (1948) studied the technology, on empirical grounds. Sykes (1962) dried single parallelepipeds; Saravacos and Charm (1962) reported critical moisture content and the drying constant for a single layer of apple dices dried at  $T_{bs} = 66^\circ C$ , RH = 13% and air velocity 1m/s. From this drying constant the effective diffusivity is  $D = 6.4 \times 10^{-5} \text{ cm}^2/\text{sec}$ . Rotstein et al. (1968) reported on diffusional aspects of single slices and rings, discussing the error involved

in using the first term of the series. Labuza and Simon (1970) studied surface tension effects, reporting an effective diffusivity  $D = 1.1 \times 10^{-5} \text{ cm}^2/\text{sec}$  when  $t_{bS} = 66^\circ\text{C}$ ,  $\text{RH} = 4-7\%$  and the air flow rate equals  $2800 \text{ ft}^3/\text{min}$ .

The present contribution seeks to obtain a dependable value of effective diffusivity on which to base an application of the above analytical prediction. To do so,  $D$  is obtained experimentally, and together with the corresponding shape factor, it is used in equations (16), (17), (28), (31), (33) and (34) to calculate the drying performance of the different shapes.

**EXPERIMENTAL**

GRANNY SMITH APPLES from Río Negro Valley, Argentina, were selected, washed, peeled and sliced to  $l_s = 1.2 \text{ cm}$ ,  $L = 0.6 \text{ cm}$ . They were sulfited by exposure to  $\text{SO}_2$  and air dried on a weighing tray. The air was filtered and heated through a heat exchanger. To secure the desired air moisture, steam was injected. The steam injection was countercurrent to insure good mixing. The air velocity was measured by a hot wire anemometer. Dry and wet bulb temperatures were continuously recorded and controlled. Air velocity was  $10 \text{ m/sec}$ ; dry bulb temperature,  $76^\circ\text{C}$ ; and relative humidity of air,  $10\%$ . A complete description of the experimental apparatus can be found elsewhere (Rotstein et al., 1968). Effective diffusivity is calculated from equation (31).

**RESULTS**

TABLE 2 reports the effective diffusivity for the first falling rate period from fourteen runs, under the conditions described. Accordingly the average value is  $D = 3.617 \times 10^{-5} \text{ cm}^2/\text{sec}$  with a standard deviation  $s = 0.145 \times 10^{-5} \text{ cm}^2/\text{sec}$ . The 95% confidence limits are  $\pm 0.084 \text{ cm}^2/\text{sec}$ . The drying performance of the different shapes considered can be compared, using this effective diffusivity. To do so, equation (31) is used to predict the drying of a cube of characteristic length  $l_s = 1.0 \text{ cm}$ . On the basis of equal weight-equal cross section, the characteristic length of the other shapes is calculated (Table 3). The characteristic length,  $l_i$ , is introduced into the shape factor  $F_i$ . Together with  $D$  it is used to predict the drying performance of the cardioid, equation (16); circle, equation (17); hexagon, equation (28); corrugated, equation (33) and epitrochoid, equation (34). The results are plotted in Figure 4.

Table 2—Effective diffusivity at  $70^\circ\text{C}$ ,  $10\% \text{ RH}$  and  $10 \text{ m/sec}$  air velocity

Run no.	$D_{ef} \text{ (cm}^2/\text{sec)} \times 10^5$
1	3.568
2	3.796
3	3.431
4	3.641
5	3.796
6	3.498
7	3.568
8	3.641
9	3.717
10	3.878
11	3.568
12	3.431
13	3.518
14	3.593

**DISCUSSION & CONCLUSIONS**

THE INTEGRATED second Fick's law is a well established model for diffusion controlled drying processes. This paper shows an approximate analytical solution. Its accuracy has been discussed elsewhere (Laura and Chi, 1965). The information obtained should be useful for design, operation, or optimization of dryers.

The effective diffusivity was determined assuming internal control. This is consistent with the approach used in the aforementioned literature, although validity of this approach at very high air velocities is now being tested by the authors.

Figure 4 shows that for the product and set of conditions

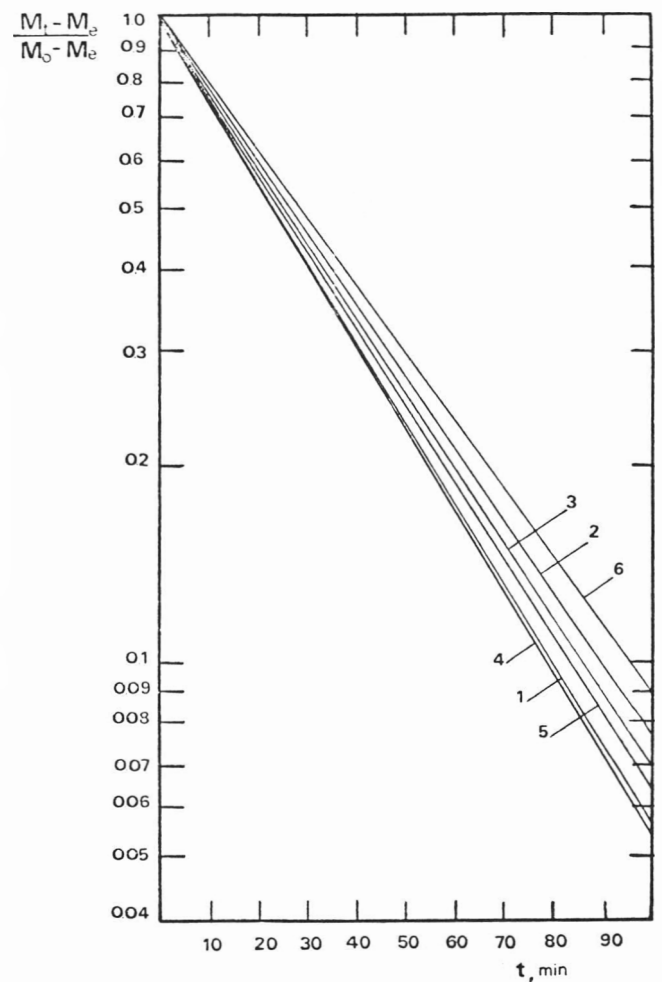


Fig. 4—Weight ratio after 100 min drying of single pieces: (1) Cardioid; (2) Circular; (3) Corrugated; (4) Square; (5) Epitrochoidal; and (6) Hexagonal cross section.

Table 3—Square of the characteristic length for each shape, based on the radius of a square of equal area

cardioid	circle	corrugated	square	epitrochoid	hexagon
$\frac{8}{3\pi} l_s^2$	$\frac{4}{\pi} l_s^2$	$\frac{4}{\pi} \sqrt{\frac{128}{129}} l_s^2$	$l_s^2$	$\frac{144}{\pi 41} l_s^2$	$\frac{8}{3\sqrt{3}} l_s^2$

involved, the effect of shape after 100 min drying cannot be overlooked. On the other hand, a good approximation to the actual performance can be obtained by assimilating the cardioid or epitrochoidal cross section to the square one. Similarly, the solutions for the corrugated and hexagonal cross section can be approximated by considering a circular one. The error by so doing is less than 5% for the cardioid and corrugated, and about 15% for the other two.

### NOTATION

A, Cross sectional area  
 $A_{op}$ , Coefficient, equation (8).  
 $A_{omp}$ , Coefficient, equation (9).  
 $b_m = (2m + 1)/2 L$   
 C, Concentration  
 D, Diffusivity  

$$F_p^i = \left( \frac{(2p + 1)^2}{4 L^2} \right)^2 + \frac{p}{l_i^2}$$
 Shape factor, where i is (i = c, cardioid, i = r, circle, i = h, hexagon, i = s, square, i = g, corrugated, i = e, epitrochoid)  
 RH, Relative humidity of air  
 i, Shape index  
 $J_0$ , Bessel function of the first kind and zero order  
 $J_1$ , Bessel function of the first kind and order one  
 L, Vertical semi-length  
 $l_i$ , Characteristic length  
 $M_0$ , Initial weight  
 $M_t$ , Weight at time t  
 r, Radius  
 s, Standard deviation  
 t, Time  
 $T_{bs}$ , Dry bulb temperature  
 V, Volume  
 $\nabla_i^2$ , Plane Laplacian operator

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## PREDICTION OF DIELECTRIC PROPERTIES IN OIL-WATER AND ALCOHOL-WATER MIXTURES AT 3,000 MHz, 25°C BASED ON PURE COMPONENT PROPERTIES

### INTRODUCTION

FURTHER UNDERSTANDING of the mechanisms of dielectric behavior in foods of diverse chemical composition and physiological states would provide a basis for optimizing current microwave food processes and could lead to the development of new processes with a unique capability for uniform heating and improved quality of product. One such possibility is seen in continuous-flow processing of liquid food systems. The electrical properties of foods appear to be extensively modified by chemical composition, which is broadly classified in proximate analysis. Although water is a major determinant of dielectric behavior in foods, its relative dielectric activity is modified by protein, lipid, carbohydrate, and ash contents in various states of solvation and suspension. Accordingly, some concepts of the relative dielectric activities of major food constituents and the ways in which they interact with water are of interest in developing predictive models for dielectric behavior of foods based on mechanisms of interaction in the microwave region. Such models would provide a basis for prediction of dielectric properties as continuous functions of frequency and temperature. These would be useful in estimating microwave heating characteristics of foods in terms of power levels, penetration depths, and frequency selection.

The literature shows several mechanisms for modification of water behavior in aqueous mixtures of constituents chemically similar to those of foods. Hasted et al. (1948) have shown that dissolved salts depress dielectric constant and elevate dielectric loss with respect to levels observed in pure water; this is due to binding of free water molecules and to increased free charge concentrations by dissolved ions. Similar effects have been observed for dissolved proteins. Mudgett et al. (1971, 1974) have shown that nonfat milk behaves as an aqueous ionic solution whose effective dissolved salts concentration is much lower than implied by total ash content. This is attributed to salts binding and complexing effects which result in a substantial fraction of undissociated milk salts.

Mudgett et al. (1974) also showed that

nonfat milk dielectric behavior could be predicted over a range of frequencies and temperatures by the Hasted-Debye models for aqueous ionic solutions, and have suggested that a similar basis of prediction may exist in aqueous food systems of low colloidal content. The properties of composite materials, however, may vary considerably from those of their individual constituents. This has been a subject of considerable interest to investigators in the electrical, chemical and biological fields. Maxwell (1881) developed a rigorous model to predict complex conductivity in uniform dispersions of spherical particles in a continuous suspending phase based on potential field theory. Rayleigh (1892) developed a similar model for long cylinders. These models recognize that electrical flux normal to a potential gradient converges in regions of high conductivity and diverges in regions of low conductivity subject to particle shape and orientation in an applied field. Fricke (1955) combined these in a single model which may be stated as:

$$\sigma^* = \frac{\sigma_c^* [\sigma_s^* (1 + xV_s) + \sigma_c^* (1 - V_s)x]}{[\sigma_c^* (x + V_s) + \sigma_s^* (1 - V_s)]} \quad (1)$$

where  $\sigma^*$ ,  $\sigma_s^*$  and  $\sigma_c^*$  are complex conductivities of the suspension, the suspended phase, and the continuous phase, respectively;  $x$  is a general form factor for particle shape and orientation; and  $V_s$  is the volume fraction of suspended particles. Fricke's equation reduces to the Maxwell model for  $x = 2$  and to the Rayleigh model for  $x = 1$ . Intermediate form factors are assumed to represent spheroidal shapes of random orientation. An interesting application of this model has been the study of intracellular differences between the vegetative and sporulative states of water and ions in *B. cereus* by Carstensen et al. (1971). Various modifications of these models used in the chemical field to measure the particulate properties of multiphase process streams have been reviewed by Meredith and Tobias (1962). These models may be expressed in terms of complex permittivity, which can then be resolved by digital computation in Cartesian or polar form

to obtain predictions of dielectric properties in such suspensions. Thus, Fricke's model may also be stated as:

$$K^* = \frac{K_c^* [K_s^* (1 + xV_s) + K_c^* (1 - V_s)x]}{[K_c^* (x + V_s) + K_s^* (1 - V_s)]} \quad (2)$$

where  $K^*$ ,  $K_s^*$ , and  $K_c^*$  are complex permittivities of the suspension, the suspended phase and the continuous phase, respectively. This form of the Fricke model is seen as a possible basis of prediction for dielectric behavior in liquid food systems of high colloidal content in which the suspended particles are of low relative dielectric activity compared with the suspending medium. The model implicitly assumes that dielectric properties of the individual phases are not significantly altered in mixture by electrochemical interaction. For such a system, then, the dielectric properties of a suspension of inert particles in an active liquid such as water should be predicted, within the range of the model, by its pure component properties. To illustrate this for lipid-water mixtures, dielectric measurements have been made for oil-water emulsions of varying composition for comparison with noninteractive Fricke model predictions.

An entirely different effect has been observed in aqueous mixtures which may also be of interest as a possible basis for modification of dielectric behavior in foods. Dielectric measurements of ethanol-water mixtures by Buck (1965) and carbohydrate-water mixtures by Roebuck et al. (1972) show dielectric loss behavior at intermediate compositions, which, based on pure component properties, is not expected. The striking difference is a synergistic loss effect where the loss of mixtures is substantially higher than the loss of either pure component. Such behavior is inconsistent with the noninteractive Fricke model. The effect is attributed to hydroxyl-water interactions which stabilize liquid structure by hydrogen bonding between the hydroxyl groups of alcohols or carbohydrates and free water molecules. In these mixtures, the pure components are highly miscible: one of the components is dissolved in the other. These results suggest that dielectric

properties of mixtures are altered by interaction of constituents in such a way that relaxation time of a mixture is shifted to some intermediate value between those of its pure components. That is, the critical wavelength of mixtures is shifted towards wavelengths of microwave processing by chemical interaction and away from regions of maximum dispersion for its pure components, so that wavelengths of measurement are closer to a new region of maximum dispersion.

Parenthetically, Grant (1965), Aaron et al. (1966) and others have observed modifications to the dielectric behavior of free water by dissolved proteins which may also be related to such effects. That is, regions of dispersion for bound water exist below that for free water and may contribute to dielectric behavior at microwave frequencies depending on the critical frequency and magnitude of such relaxations. The general effect of dissolved proteins is to depress the dielectric constant and elevate the dielectric loss with respect to pure water. However, such effects are believed to be of second order importance at frequencies of interest in microwave processing, particularly when correction is made for volume exclusion effects associated with the colloidal behavior of proteins. Additional mechanisms of interaction with water in heterogeneous mixtures are noted by Schwan and Cole (1960), de Loor (1968) and others for double layer and surface conductivity effects of inclusions which may give rise to a distribution of relaxation times depending on size and shape of the inclusions. While the magnitude of such dispersions may be greater than observed for bound water relaxations, their critical frequencies are generally much lower. However, the possibility is seen that dispersions associated with surface conductivity could conceivably extend into the microwave region in foods of complex biological composition and structure. These observations suggest that the critical wavelength of such "mixtures" may also be intermediate to those of its major constituents.

Interestingly enough, dielectric constant in methanol-water and ethanol-water mixtures is closely predicted by the noninteractive Fricke model. But a careful review of the literature shows no predictive model for synergistic loss behavior based on pure component properties. However, approximate values of the Debye parameters which determine dielectric behavior in pure polar liquids with single relaxation times were found for water, methanol and ethanol in publications by Collie et al. (1948), Hasted and Roderick (1958) and Buck (1965). These include the static dielectric constant ( $K_s$ ), the optical dielectric constant ( $K_o$ ), and the critical wavelength ( $\lambda_s$ ), which are shown in Table 1.

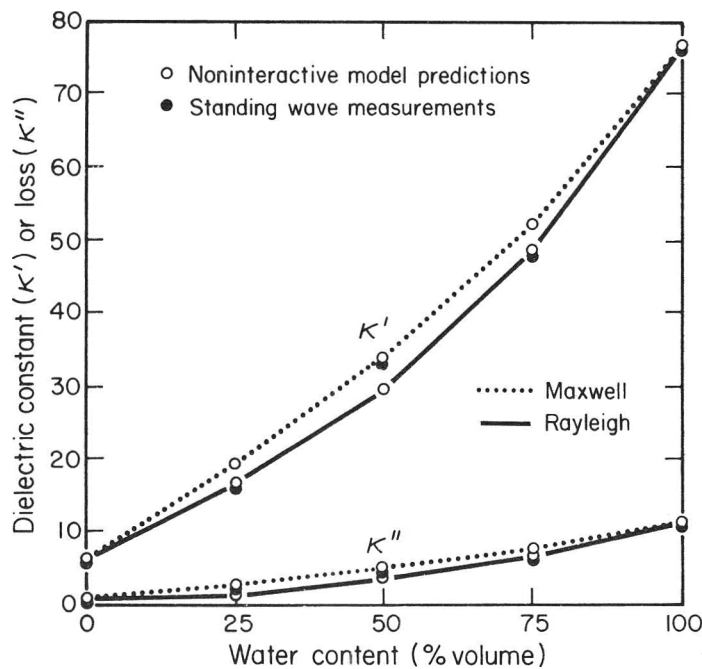


Fig. 1—Predicted vs. measured dielectric properties in oil-water emulsions at 3,000 MHz, 25°C.

A model was then sought to predict observed loss behavior in alcohol-water mixtures. One approach was to assume that alcohol-water interactions result in modification of pure components to give intermediate values of the Debye parameters, which may be predicted by the noninteractive Fricke model. Intermediate values of the static dielectric constant, optical dielectric constant and critical wavelength ( $K_{si}$ ,  $K_{oi}$  and  $\lambda_{si}$ ) thus obtained by substitution from pure component Debye parameters in the noninteractive Fricke model were then substituted in the Debye models for polar liquids with single relaxation times to obtain predictions of dielectric constant and loss in alcohol-water mixtures for comparison with dielectric measurements. The resulting models, an empirical combination of the Fricke and Debye models for dielectric behavior, are designated in further

discussion as the interactive Fricke model:

$$K' = \frac{(K_{si} - K_{oi})}{1 + (\lambda_{si}/\lambda)^2} + K_{oi} \quad (3)$$

$$K'' = \frac{(K_{si} - K_{oi})(\lambda_{si}/\lambda)}{1 + (\lambda_{si}/\lambda)^2} \quad (4)$$

## EXPERIMENTAL

### Oil-water emulsions

Olive oil-water emulsions at water contents of 0, 25, 50, 75 and 100% total volume were prepared as follows: 98.5 of olive oil was heated to 70°C, and 1.5 ml Span 60 and 3.5 ml Tween 60 were added; the mixture was magnetically stirred for 15 min at 70°C. Water heated separately to 70°C was then added in the necessary proportions to the mixture in a blender at low-speed agitation for 1 min, followed by high-speed agitation for 3 min to obtain the compositions indicated. Mixtures were then cooled to 25°C in capped Erlenmeyer flasks at ambient temperature.

### Alcohol-water mixtures

Alcohol-water mixtures at water contents of 0, 25, 50, 75 and 100% total volume were prepared by mixing alcohols and water in the necessary proportions in a 100 ml graduated cylinder with magnetic stirring. No correction was made for volume shrinkage of 1–2% observed in mixing.

### Chemicals

Olive oil of commercial grade was purchased from the Gloria Packing Co., Boston, Mass.; absolute ethanol of reagent grade from U.S.

Table 1—Pure component properties of alcohols and water at 25°C

Liquid	$K_s$	$K_o$	$\lambda_s$
Methanol <sup>a</sup>	32.4	5.5	9.9 cm
Ethanol <sup>b</sup>	24.4	4.2	31.9
Water <sup>c</sup>	78.5	5.5	1.6

<sup>a</sup> Hasted and Roderick (1958)

<sup>b</sup> Buck (1965)

<sup>c</sup> Collie et al. (1948)

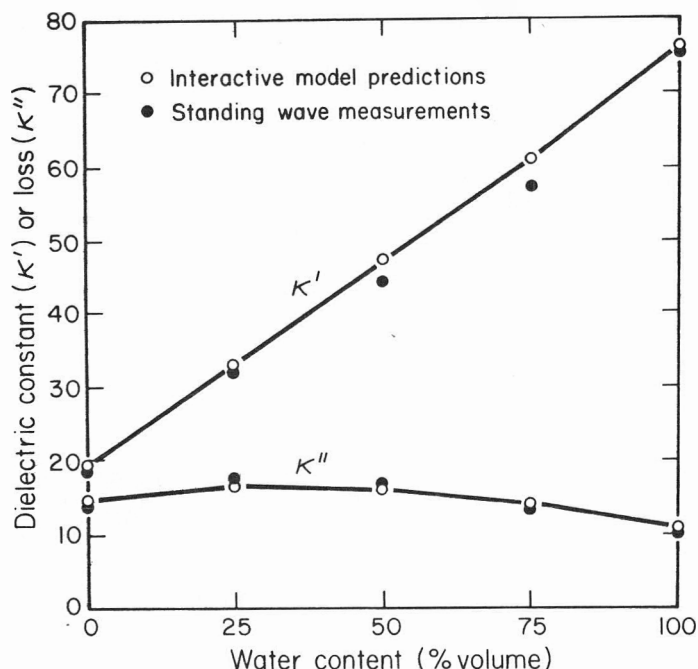


Fig. 2—Predicted vs. measured dielectric properties in methanol-water mixtures at 3,000 MHz, 25°C.

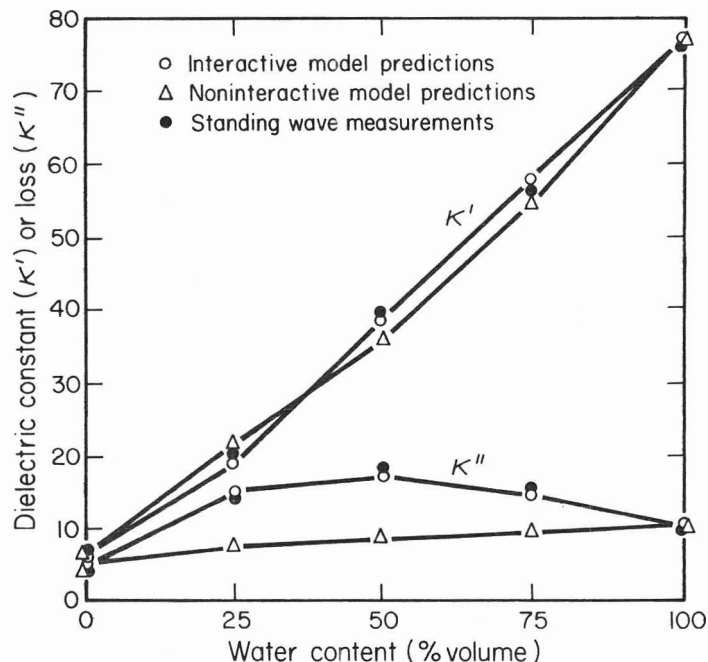


Fig. 3—Predicted vs. measured dielectric properties in ethanol-water mixtures at 3,000 MHz, 25°C.

Industrial Chemicals Co., New York, N.Y.; and absolute methanol of reagent grade from Fisher Scientific Co., Fair Lawn, N.J. Span 60 and Tween 60 were purchased from Atlas Chemical Industries, Wilmington, De.

#### Dielectric measurements and computations

Standing wave measurements at 3,000 MHz, 25°C were made for oil-water emulsions and methanol- and ethanol-water mixtures for water contents of 0, 25, 50, 75 and 100% total volume. Duplicate measurements were made for all samples and were reproducible to within 2%. Pace et al. (1968) have previously described the method (Roberts and Von Hippel, 1946) and equipment.

Dielectric properties from standing wave measurements and model predictions (Maxwell, 1881; Rayleigh, 1892) were calculated by computer programs written in complex notation for the IBM 360 and 1130 computers.

## RESULTS & DISCUSSION

MEASURED dielectric properties for oil-water emulsions of varying composition are compared with predictions by the noninteractive Fricke model at 3,000 MHz, 25°C, in Figure 1. Dielectric constants are generally within 5% of Rayleigh and 10% of Maxwell predictions while dielectric losses are within 5% of both Maxwell and Rayleigh predictions at all compositions. Measured dielectric properties for methanol-water mixtures of varying composition are compared with predictions by the interactive Fricke model in Maxwell form at 3,000 MHz, 25°C in Figure 2. Both dielectric constants and

losses are within 5% of interactive model predictions for all compositions. Similar results were obtained for ethanol-water mixtures, as shown in Figure 3. To illustrate the difference between interactive and noninteractive forms, noninteractive Maxwell predictions are also shown in Figure 3. It is seen that dielectric constants agree about as closely with noninteractive as they do with interactive model predictions, but dielectric losses are seen to vary from noninteractive model predictions by as much as 100%. Model comparison also illustrates the synergistic loss effect observed in mixtures of interactive constituents with single relaxation times. A quantitative theoretical basis cannot be given at this time for these results, but the interactive

form of the Maxwell model gives close predictions of dielectric behavior at 3,000 MHz, 25°C for two different alcohol-water mixtures. Similar modifications of dielectric behavior may also exist in liquid food systems with high levels of dissolved sugars or carbohydrates.

A tentative physical-chemical basis for prediction of dielectric behavior in liquid food systems, which may be related to chemical composition in terms of proximate analysis, is now seen for three possible mechanisms which have been observed to modify the relative contribution of water to overall dielectric activity. These are summarized in Table 2. The effect of dissolved salts is to depress dielectric constant and elevate dielectric loss with respect to levels observed in

Table 2—Observed modifications to dielectric behavior of water in various aqueous mixtures<sup>a</sup>

Aqueous mixture	Modification	Model behavior	Related chemical constituents
Water	—	Debye	Moisture
Nonfat milk	Dissolved ions	Hasted-Debye	Ash, protein
Oil-water	Suspended colloids	Fricke	Lipid, protein, carbohydrate
Alcohol-water	Hydroxyl interactions	Maxwell-Debye	Carbohydrate

<sup>a</sup> Based on work by Mudgett et al. (1974), and on observations made in this work.



pure water. Such behavior in nonfat milk has been predicted by the Hasted-Debye models and appears to be related primarily to water content and dissolved salts levels represented by ash content modified to reflect salts binding and complexing effects. The major effect of protein content in aqueous mixtures is believed to be similar to that of dissolved salts because of surface charge effects resulting from ionization of free carboxyl, amine and sulfhydryl substituents which lead to binding of free water molecules and increased free charge density in solution. However, substantial volume fractions of dissolved proteins may also exist in a colloidal state, which suggests that the relative dielectric activity of dissolved proteins may vary considerably in specific systems.

The apparent effect of suspended colloids is the volumetric exclusion of dielectrically active water from aqueous mixtures by colloidal materials of low dielectric activity, thus depressing dielectric constant and loss. Such behavior has been demonstrated for oil-water mixtures and has been predicted by the Fricke model, in terms of complex permittivity, for intermediate compositions based on pure component properties. Similar behavior is expected in liquid food systems of high lipid content; for example, milk of high butterfat content or fruit juices with high pulp levels. Finally, the effect of dissolved hydroxyl substituents is to modify dielectric behavior in aqueous mixtures through hydroxyl-water interactions which appear to stabilize liquid structure by hydrogen bonding and to shift relaxation times of mixtures away from those of their pure components to intermediate levels which give rise to synergistic loss effects at microwave frequencies. Such effects have been observed

in alcohol-water mixtures and have been predicted from pure component properties by an empirical correlation involving the Maxwell and Debye models. Similar behavior is possible in liquid food systems with high levels of dissolved carbohydrates. Conceivably, one or more of these mechanisms may be operative in a particular food system.

Such mechanisms for modification of the relative dielectric activity of water in aqueous mixtures appear to offer a basis of prediction for dielectric behavior in liquid food systems related to chemical composition, which could be useful in estimating microwave heating characteristics over a continuous range of frequencies and temperatures. Similar mechanisms of dielectric behavior in solid foods are seen in terms of chemical composition subject to further modifications due to the effects of biological structure. A physical-chemical basis for prediction of dielectric behavior in solid foods is currently being investigated and will be reported in a subsequent paper.

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HEIKKI PYYSALO and TAINA KUUSI

Food Research Laboratory, Technical Research Centre of Finland, SF-02150 Otaniemi, Finland

## A Research Note

PHENOLIC COMPOUNDS FROM THE BERRIES OF  
MOUNTAIN ASH, *Sorbus aucuparia*

## INTRODUCTION

THE BERRIES of Mountain Ash, *Sorbus aucuparia* L., are rich in vitamins; for instance they contain 40–60 mg ascorbic acid per 100g berries (Benk, 1960; Kuusi, 1969). However, they enjoy only limited use because of their bitter taste. The bitterness is partly due to glucosidic precursor of sorbic acid, trans-3- $\beta$ -D-glucopyranosyloxy-5-hexanolide (Letzig, 1964; Tschesche et al., 1971; Pyyssalo and Kuusi, 1971). Mountain Ash berries also contain the phenols cyanidin-3-galactoside and/or cyanidin-3,5-diglucoside, quercetin, isoquercitrin, and rutin (Willstätter and Bolton, 1917; Fouassin, 1956; Shnaidman et al., 1971).

Certain polyphenols are known to be bitter, and the identification, regulation and removal of any such bitter substances could prove important in the commercial use of Mountain Ash, particularly in northern lands where the tree flourishes. Accordingly, the main polyphenolic components of the berries were identified and tested for bitterness.

## MATERIAL &amp; METHODS

COMMON METHODS in polyphenol chemistry were applied (Mabry et al., 1970). The juice was pressed from 5 kg red and ripe berries which were collected at the beginning of September before the first frost. These were stored for a few weeks at  $-30^{\circ}\text{C}$  and thawed before pressing. The juice was diluted with a large amount of acetone and a brown precipitate formed. After separation of the precipitate the acetone was evaporated; lipid material was extracted from the residue into chloroform and light petroleum ether.

The polyphenols were then extracted into ethyl acetate from the liquid remaining and after evaporation of the ethyl acetate, 150g of a dark brown syrup containing about 10% poly-

phenols was obtained. The polyphenol determinations were made by the Folin-Denis method (AOAC, 1965); commercial chlorogenic acid or commercial malvidin-3,5-diglucoside purified by paper chromatography were used in the preparation of standard curves. The anthocyanins were only slightly soluble in ethyl acetate and were separated by precipitation with neutral lead acetate (Geissman, 1962) and by ion exchange chromatography (Fuleki and Francis, 1968; Timberlake and Bridle, 1971).

Of the final syrup 12.5g were distributed into portions by counter-current method (Hietala, 1960), using as solvent system butanol-acetic acid-water 16:1:10, the two phases 1:1, upper (butanolic) phase as mobile, i.e., 1 ml per minute. An instrument with 300 separation units, each with a volume of 13.5 ml, was used. The separation was followed by UV detectors at 254 and 280 nm; fractions of 30 ml were collected and quantitative polyphenol determinations were made. The fractions from counter-current distribution still contained several components but the method served to enrich the polyphenols. Another useful phase system was Sorensens phosphate buffer solution, pH 5.9–ethyl acetate (the two phases 1:1), ethyl acetate as mobile (Corse et al., 1965). Counter-current distribution fractions were further fractionated by two-dimensional paper chromatography and the compounds were then identified by paper chromatographical data, Hoepfner reagent i.e., 5% acetic acid and 5% sodium nitrite, 1:1 (Swain, 1953; Rivas and Luh, 1968), diazotized sulfanilic acid (DSAA) (Cramer, 1954), UV spectra, and by studying the products of hydrolysis after boiling 1 hr in 1N hydrochloric acid or 5% trifluoroacetic acid. Trans-chlorogenic acid, quercetin and rutin were also identified as trimethylsilyl ethers and by gas-liquid chromatography (Keith and Powers, 1966) and by NMR spectra (Table 2). The sugars from hydrolysis were identified as TMS ethers by GLC with 4% SE 52, 1.8m glass column, at 150–170 $^{\circ}\text{C}$  and also by TLC with methyl-ethyl-ketone-methanol-water (60:12:18) as solvent system and phosphoric acid-aniline as spray reagent (Stahl, 1962).

## RESULTS

TABLE 1 presents results of two-dimensional paper chromatography of phenolic compounds of Mountain Ash berries.

The berries were found to be rich in hydroxycinnamic acids, the main component of which was identified by shifts and coupling constants in the NMR spectrum as trans-chlorogenic acid (Table 2). Caffeic, p-coumaric and ferulic acids were found to be identical with corresponding commercial acids, which were confirmed by NMR spectra as trans-isomers. The main red pigment proved to be cyanidin-3-galactoside. Only traces of anthocyanins 13 and 14 were found, making their identification difficult.  $R_f$  values (Table 1) suggest that they were cyanidin-3-arabinoside and cyanidin-3,5-diglucoside. Through hydrolysis a mixture of compounds 12–14 in addition to glucose and galactose, traces of arabinose were detected in GLC. The TMS ether of rutin was isolated using preparative gas chromatography with 5% SE, 30m column, at 265 $^{\circ}\text{C}$ . The silylations were made with trimethylchlorosilane and hexamethylenedisilazane in pyridin (Mabry et al., 1964). Enough quercetin was collected from paper chromatograms to record NMR spectrum, which was identical with that in literature (Mabry et al., 1970). Cyanidin was obtained after boiling leucocyanidin for 1 hr in 1N HCl (Swain and Hillis, 1959).

The total amount of polyphenols obtained from fresh berries was 0.45%. This includes the 0.15% of anthocyanins. Compounds 1, 11, 16 and 17 form about 80% of the total polyphenols. In simple preliminary organoleptical tests neither the precipitate formed in acetone dilution nor the extracts into chloroform and petroleum ether and into ethyl acetate

Table 1— Two-dimensional paper chromatography of phenolic compounds of Mountain Ash berries<sup>a</sup>

No	Compound	R <sub>f</sub> 1 <sup>1</sup>	R <sub>f</sub> 2 <sup>1</sup>	UV	UV + NH <sub>3</sub>	Hoepfer reagent	DSAA	UV-spectrum			AICl <sub>3</sub> shift (nm)	Hydrolyse products
								λ max (nm)	λ min (nm)	λ max (nm)		
1	trans-Chlorogenic acid	0.60	0.56	BF	G	BrO	BrO	300	330	267 <sup>c</sup>	+	caffeic & quinic acid
2	cis-Chlorogenic acid	0.60	0.70	BF	G	BrO	BrO	302	329	267 <sup>c</sup>	+	caffeic & quinic acid
3	trans-Neochlorogenic acid	0.55	0.58	BF	G	BrO	BrO	300	333	268 <sup>c</sup>	+	caffeic & quinic acid
4	cis-Neochlorogenic acid	0.55	0.65	BF	G	BrO	BrO	302	330	268 <sup>c</sup>	+	caffeic & quinic acid
5	Isochlorogenic acid isomer <sup>k</sup>	0.65	0.43	BF	G	BrO	BrO	303	332	269 <sup>c</sup>	+	caffeic & quinic acid
6	trans-Caffeic acid	0.80	0.25	BF	BG	R	R	300	328	265 <sup>c</sup>	+	—
7	trans-Ferulic acid	0.88	0.75	BF	BG	Br	DV	295	321	261 <sup>c</sup>	—	—
8	Feroy/quinic acid ester	0.7	0.75	BF	BG	Br	DV	304	331	266 <sup>c</sup>	—	Ferulic & quinic acid
9	trans-Coumaric acid	0.90	0.45	—	DV	YO	BR	297	312	249 <sup>c</sup>	—	—
10	p-Coumaryl/quinic acid ester	0.65	0.75	—	DV	YO	BR	299	326	268 <sup>c</sup>	—	Coumaric & quinic acid
11	Cyanidin-3-galactoside	0.37	0.22 <sup>b</sup>	—	—	—	—	532 <sup>d</sup>	—	—	27	Cyanidin & galactose
12	Cyanidin-3-glucoside	0.36	0.23 <sup>b</sup>	—	—	—	—	530 <sup>d</sup>	—	—	18	Cyanidin & glucose
13	Cyanidin-glycoside	0.45	0.33 <sup>b</sup>	—	—	—	—	541 <sup>e</sup>	—	—	10	Cyanidin & arabinose
14	Cyanidin-3,5-diglycoside	0.28	0.1 <sup>b</sup>	F	—	—	—	543 <sup>e</sup>	—	—	7	Cyanidin
15	Leucocyanidine	0.91	0.01 <sup>e</sup>	Y	—	Pink with vanillin reagent <sup>f</sup>	—	276 <sup>h</sup>	—	—	—	Cyanidin
16	Quercetin	0.6 <sup>i</sup>	0.01 <sup>e</sup>	Y	Y	—	—	257	259	290 <sup>h</sup>	16 <sup>j</sup>	—
17	Rutin	0.45 <sup>i</sup>	0.45 <sup>e</sup>	D	Y	—	—	360	360	308 <sup>h</sup>	17 <sup>j</sup>	Quercetin, glucose, rhamnose
18	Hyperoside	0.5 <sup>i</sup>	0.45 <sup>e</sup>	D	Y	—	—	257	357	315 <sup>h</sup>	17 <sup>j</sup>	Quercetin, galactose

a R<sub>f</sub>1 = butanol-acetic acid-water (4:1:5), upper phase. R<sub>f</sub>2 = 2% acetic acid; B = blue; F = fluorescence in UV light; Y = yellow; D = dark; V = violet; Br = brown; O = orange; R = red; BR = brick red; G = green.  
 b butanol-1-n hydrochloric acid, (1:1), upper phase  
 c 94% ethanol  
 d 0.01% hydrochloric acid in methanol  
 e 15% acetic acid  
 f vanillin-hydrochloric acid (Swain and Hillis, 1959)  
 g 0.01% hydrochloric acid in 94% ethanol  
 h in methanol  
 i butanol-acetic acid-water (3:1:1), upper phase  
 j from 257 or 259 nm  
 k for names see Corse et al. (1965)  
 l values in lit., see e.g., Geissman (1962); Luh et al. (1967); Rivas and Luh (1968).

Table 2—δ-values in NMR spectra of silylated polyphenols in CCl<sub>4</sub>, TMS as internal standard

	H2 <sup>a</sup>	H3	H4	H5	H6	Hα	Hβ	H8	H2'	H5'	H6'
Trans-Chlorogenic acid	2.1m	4.9m	3.66m	4.0m	2.1m	6.04d	7.45d		6.9m		
Quercetin	3.47m				6.16d	J=15.8 Hz		6.46m	7.6m	6.48m	7.6m
Rutin					6.18d			6.46m	7.47	6.87	7.47
	10 rhamn. prot. 3.5m Rhamn. 3 methyl prot. 0.81m Rhamn. H1 4.3s										

<sup>a</sup> For the numbering of protons see e.g., Mabry, et al. (1964).

exhibited a strong bitter taste. Rather, the residue had the typical strong bitter taste. This residue contained no polyphenols but a considerable amount of trans-3- $\beta$ -D-glucopyranosyloxy-5-hexanolide. The polyphenols eluted from paper chromatograms and counter-current distribution fractions did not exhibit a strong bitter taste. However, physiological and pharmacological effects have been reported for hydroxycinnamic acids (Chassevent, 1969) and many of the acids are known to be precursors of volatile aroma compounds (Steinke and Paulson, 1964).

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## A Research Note COLLAGEN CHARACTERISTICS OF PRONGHORN ANTELOPE AND SHEEP MUSCLE

### INTRODUCTION

MEAT from antelope is very tender. After aging and cooking the extreme tenderness (W-B shear on 1.27 cm cores = 1.2 kg) of the meat gives a "mushy" consistency (Field et al., 1972). Cooked lamb meat, which is usually considered to be tender, has average W-B shear values of 2.38–4.74 kg on 1.27 cm cores (Field, 1971). On the molecular level, meat tenderness depends partially on the number of crosslinkages present in the intramuscular collagen molecule (Kruggel and Field, 1971). In the present study, soluble intramuscular collagen was isolated from antelope and sheep muscle to determine if the difference in tenderness between the two species is related to differences in collagen intramolecular crosslinkages.

### EXPERIMENTAL

THE LONGISSIMUS MUSCLE from each side of five yearling antelope, five older antelope, two lambs and two ewes (7 yr old) was used. The carcasses were aged 7 days at 2°C before removal of the muscle. Intramuscular collagen was obtained from the muscle and purified by the procedure of Kruggel and Field (1971). A guanidine hydrochloride solution was prepared according to Kruggel and Field (1971) and a modification of Mohr and Bendall's (1969) extraction procedure was used to obtain the guanidine hydrochloride soluble intramuscular collagen (GSIC). Hydroxyproline content of the purified, lyophilized GSIC, determined according to the hydrogen peroxide procedure of Dahl and Persson (1963) was 11.50–12.92%. Since GSIC contains an appreciable amount of crosslinks, it was chosen for this study. However, it only represented approximately 1% of the total intramuscular collagen.

Gel-disc electrophoresis was performed on the denatured GSIC according to Clark and Veis (1968) as modified by Pfeiffer et al. (1972), except that a concentration of 5 mg collagen per ml of 0.01N acetic acid was used. After staining the gels and scanning, the peak areas of the  $\alpha$ -,  $\beta$ - and  $\gamma$ -components were obtained by use of a compensating polar planimeter. Percent of the three chain components was then calculated as percent of the total area.

Percent moisture in the longissimus muscle of each sample was determined by drying a small portion to a constant weight. Total collagen in the longissimus muscle was determined according to Pfeiffer et al. (1972) and reported on a dry weight basis.

### RESULTS & DISCUSSION

INTRAMUSCULAR COLLAGEN from yearling antelope had almost twice as much collagen solubilized by the guanidine hydrochloride solution as did the intramuscular collagen from the older antelope (Table 1). This may be explained on the basis that the intramuscular collagen in the yearling antelope is not as mature as the intramuscular collagen in the older antelope, thus not as highly crosslinked and more soluble. Goll et al. (1962) found that beef collagen solubility decreased significantly with advancing maturity even though the collagen content was not different. They attributed this to more frequent crosslinks among the tropocollagen molecules of collagen from the older age group. Hill (1966) found that the percent of intramuscular collagen solubilized by 1/4-strength Ringer's solution decreased as chronological age increased. In the present study, the same soluble collagen effect was found in the sheep samples as in the antelope samples. Lambs contained about twice the amount of GSIC as old ewes.

In the yearling antelope and lamb groups, approximately the same amount of intramuscular collagen was solubilized by the guanidine hydrochloride solution. The older antelope and ewe groups also showed a similar percentage of intramuscular collagen solubilized.

This study shows that the pronghorn antelope has very little total collagen within its muscles when compared to

domestic sheep (Table 1). Therefore, animals capable of running at high speeds for long distances do not necessarily contain more connective tissue within their muscles than domestic animals, some of which have very little exercise. These data support the work of Rusk et al. (1931) who showed that muscles from exercised steers have less collagen than steers which received very little exercise. According to Field et al. (1972) total lean and fat from carcasses of mature male and female antelope contain 1.7 and 1.4% collagen on a wet basis, respectively. Comparable figures obtained in this study on four 7-yr-old ewe carcasses gave an average of 2.69% collagen in the total fat and lean.

The lambs had a smaller percentage of collagen present than the old ewes. Cross et al. (1973) also reported increased collagen content in beef longissimus muscle as age increased from 483 to 4087 days. It is well established that the increase in intramuscular collagen is not linear with age. Wilson et al. (1954) and Goll et al. (1963) have shown that very young calves also have high amounts of intramuscular collagen. Increases in intramuscular collagen in very old animals may be explained according to Schaub (1963). He found that, in old rats, new collagen was being formed in the aging striated muscle at a similar rate as that in young animals. It is probable that the 7-yr-old ewes in this study were old enough for additional collagen formation to occur. The older antelope may not have reached the age to allow additional collagen formation. It is

Table 1—Means and standard errors for characteristics of antelope and sheep longissimus muscles

Characteristic	Antelope		Sheep	
	Yearlings	Old does	Lambs	Old ewes
Soluble intramuscular collagen, % <sup>a</sup>	1.10 ± 0.27	0.57 ± 0.24	1.00 ± 0.37	0.41 ± 0.06
Moisture, %	73.60 ± 0.57	73.52 ± 0.18	74.88 ± 0.05	71.82 ± 0.37
Total collagen, % <sup>b</sup>	0.94 ± 0.17	0.84 ± 0.07	1.90 ± 0.07	3.22 ± 0.41

<sup>a</sup> Soluble intramuscular collagen expressed as a percent of total intramuscular collagen

<sup>b</sup> Total intramuscular collagen expressed as a percent of the moisture free muscle

Table 2—Means and standard errors for percentage of collagen chain components obtained from gel-disc electrophoresis<sup>a</sup>

Collagen chain component	Antelope		Sheep	
	Yearlings	Old does	Lambs	Old ewes
$\alpha$	62.49 ± 3.22	58.63 ± 0.31	73.23 ± 4.18	79.19 ± 4.50
$\beta$	27.47 ± 2.73	29.31 ± 0.04	21.91 ± 5.15	11.04 ± 3.97
$\gamma$	10.04 ± 0.55	12.06 ± 0.32	4.86 ± 0.98	9.77 ± 0.53

<sup>a</sup> Values are percent of total peak area

also possible that additional collagen formation does not occur in muscles of animals like antelope where there are high levels of physical activity. Percent moisture in the lean of all sheep and antelope samples was reflective of the fact that the longissimus muscles and carcasses of all animals were practically devoid of fat.

In the gel-disc electrophoresis study of denatured GSIC, a lower percentage of  $\alpha$ -chain and a higher percentage of  $\beta$ -component was found in the yearling and older antelope muscle than in the lamb and ewe muscle (Table 2). Therefore, collagen within the antelope muscle is more highly crosslinked than sheep muscle. Antelope muscle should be considerably tougher than sheep muscle when crosslinking of collagen alone is considered (Kruggel and Field, 1971). However, earlier studies (Field, 1971) show that antelope muscle is very tender. The percent of collagen present in the antelope longissimus is much lower than that in the sheep. It appears that meat tenderness not only depends on the amount of crosslinking in the tropocolla-

gen molecule (Hill, 1966; Carmichael and Lawrie, 1967; and Kruggel and Field, 1971) but also on the amount of highly crosslinked collagen present (Loyd and Hiner, 1959; Cover et al., 1957).

The denatured GSIC from the old ewes contained a larger percentage of  $\alpha$ -chain and a lower percentage of  $\beta$ -component than that from the lamb samples (Table 2). This may be explained by the results of Schaub (1963) in that, in old striated muscle, new collagen is formed. This newly formed collagen would naturally have less crosslinkages than the more mature collagen found in lamb muscle.

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## A Research Note

# PROTECTIVE EFFECT OF FORTIFIED SKIM MILK AS SUSPENDING MEDIUM FOR FREEZE DRYING OF DIFFERENT LACTIC ACID BACTERIA

### INTRODUCTION

IN AN EARLIER study (Sinha et al., 1970), it was shown that reconstituted skim milk fortified with ascorbic acid, thiourea and ammonium chloride improved cell survival of freeze-dried *Streptococcus lactis* C-2. The present paper reports the use of the above suspending medium for studying the survival of several other lactic acid bacteria.

### EXPERIMENTAL

23 DIFFERENT CULTURES of lactic acid bacteria comprising streptococci, leuconostocs and lactobacilli were grown in appropriate growth media. Washed cells of the test organism were resuspended in reconstituted skim milk (10%) fortified with ascorbic acid, thiourea and ammonium chloride (0.5% each). 0.2 ml of the cell suspension was transferred to each ampoule. Freeze drying was carried out in a Centrifugal Freeze Dryer (M/S Edwards High Vacuum Co., England, Model LT5/M200) according to the procedure described earlier (Sinha et al., 1972). At the end of drying, half the number of ampoules of the particular test culture were sealed under vacuum (chamber pressure 20–25 microns of Hg) and the other half were sealed in air. The ampoules were then stored at 30°C for 2 months. The viability of the freeze-dried cells was determined before and after freeze drying as well as after storage.

### RESULTS & DISCUSSION

RESULTS on the use of fortified skim milk as suspending medium with different lactic acid bacteria are presented in Table 1. In general, higher cell survivals (80–100%) were noticed when freeze-dried cultures were examined for viable counts after vacuum sealing as compared to air sealed conditions. Among streptococci, low cell survival was observed in *S. cremoris* and in two strains of *S. thermophilus* (Italy and Hansens), especially after storage in air sealed ampoules, while among lactobacilli, *L. bulgaricus* (Yb and LBW) and *L. lactis* (a local strain) were

Table 1—Protective effect of fortified skim milk as suspending medium on various lactic acid bacteria<sup>a</sup>

Sl no.	Microorganisms	Survival (%)			
		After freeze-drying <sup>b</sup>		After storage <sup>c</sup>	
		Vacuum	Air	Vacuum	Air
1.	<i>Streptococcus lactis</i> C-10	87.3	84.0	48.8	10.8
2.	<i>Streptococcus lactis</i> 496	99.9	82.3	54.1	10.0
3.	<i>Streptococcus lactis</i> ML2	99.4	89.7	60.8	37.2
4.	<i>Streptococcus diacetilactis</i> DRC-1	100.0	96.4	60.7	11.1
5.	<i>Streptococcus diacetilactis</i> DRC-2	90.9	90.9	45.5	25.1
6.	<i>Streptococcus diacetilactis</i> DRC-3	73.3	72.3	50.7	21.7
7.	<i>Streptococcus diacetilactis</i> (local strain)	98.3	97.1	90.8	40.8
8.	<i>Streptococcus cremoris</i> R6	70.5	59.2	0.3	0.03
9.	<i>Streptococcus cremoris</i> HP	38.9	28.0	7.0	0.6
10.	<i>Streptococcus cremoris</i> C-13	30.6	22.6	6.9	1.2
11.	<i>Streptococcus faecalis</i> S-30	93.9	92.1	77.9	52.1
12.	<i>Streptococcus faecalis</i> 190	99.3	80.1	25.8	10.7
13.	<i>Streptococcus citrophilus</i> 209	90.9	84.4	60.0	23.9
14.	<i>Streptococcus thermophilus</i> YS	95.5	94.4	79.0	53.1
15.	<i>Streptococcus thermophilus</i> Italy	76.6	72.1	9.8	5.5
16.	<i>Streptococcus thermophilus</i> Hansens	92.3	88.1	58.9	7.9
17.	<i>Leuconostoc citrovorum</i> ATCC	96.6	81.6	64.5	39.6
18.	<i>Leuconostoc dextranicum</i> 551	87.2	78.8	65.1	16.6
19.	<i>Lactobacillus bulgaricus</i> Yb	13.7	6.8	6.1	1.2
20.	<i>Lactobacillus bulgaricus</i> LBW	19.8	17.4	9.3	2.9
21.	<i>Lactobacillus lactis</i> (local strain)	7.2	3.9	3.6	1.6
22.	<i>Lactobacillus casei</i> 300	99.5	98.8	49.2	32.1
23.	<i>Lactobacillus bifidus</i> 2797	93.7	92.1	57.6	53.5

<sup>a</sup> Condition of sealing ampoules after freeze drying: Vacuum and air

<sup>b</sup> Freeze-drying time: 4 hr

<sup>c</sup> Freeze dried cultures were stored for 2 months at 30°C.

susceptible to the stresses of freeze drying as is evident by low cell survivals. In *L. bifidus* and *L. casei*, however, the percentage survival during freeze drying as well as after storage was comparatively high. Gavin (1968) reported higher cell survival of short rods as compared to the larger rod forms of lactobacilli.

Among the several lactic acid bacteria examined in the present study, *S. faecalis* S-30, *S. thermophilus* Ys and *L. bifidus* 2797 exhibited maximum cell survival after freeze drying and storage with the use of fortified skim milk as suspending

medium. The low cell survival noted in *S. cremoris* may be due to its morphological characteristics than to its strain sensitivity to the freeze-drying process (Schubert, 1959). The inability of the components of the cell to withstand stresses at various stages of freeze drying (Proom, 1951) may, perhaps, explain the low cell survival of *L. bulgaricus* and *L. lactis* in the present study. Higher cell survival noticed in fortified skim milk with several cultures of lactic acid bacteria, seems to indicate that the mechanism of protection offered by the suspending medium (Mori-

<sup>1</sup>FAO Expert, Post Box 1966, Lusaka, Zambia

chi, 1972) may be similar in all the organisms. The present studies have also shown that freeze-dried bacterial cells sealed in ampoules are susceptible to oxygen, irrespective of the physiological characteristics of the cultures. The earlier report of Obayashi et al. (1961) of higher survival of *L. bifidus* in air than in vacuum is in contradiction to the current observations presented in this paper.

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## A Research Note

### LONG-TERM STORAGE OF CELLS OF *Candida utilis*: A NOTE ON THEIR NUTRITIONAL EVALUATION

#### INTRODUCTION

THE STABILITY of single-cell protein is a problem of great importance when we consider that these biomasses should, ideally, be used in a large-scale production of protein-rich mixtures with no special storage requirements. Recently, Yanez et al. (1972) have pointed out the decrease in the biological value of *Candida utilis* cells when stored for a year in paper bags at room temperature. This preliminary research note shows that the low biological value of long-stored cells is not corrected by the simultaneous addition of casein and DL-methionine. Otherwise, chemical and histo-pathological data do not suggest the presence of toxic compounds; indeed, intestinal disturbances were the solely noticed ill-effects.

#### EXPERIMENTAL

THE CELLS were grown on molasses and salts and were stored in our laboratories for 3–4 yr under uncontrolled conditions (room temperature ranging from 10–39°C; relative humidity usually higher than 70%). The sample was a fine light-colored powder and was free of bad odors.

##### Chemical analysis

Total nitrogen was determined according to AOAC (1970); ribonucleic acid, according to Webb and Levy (1967); moisture by drying at 105°C to constant weight; amino acids with a Beckman amino acid analyser, model 121; tryptophan, according to Pinto (1972), after treating by toluene.

##### Feeding test

The feeding test was carried out by the procedure described by Campbell (1963). The recommended 10% protein content of the diet was achieved by mixing 6.25% casein and 11.5% *Candida utilis* cells. The growth assay was controlled by the simultaneous feeding of another group of rats in completely identical conditions except for the substitution of the 11.5% old cells by recently harvested cells of *C. utilis*; the final diet contained the same crude protein content (10%).

##### Histological analysis

Data from Silveira (1967) and Guanabara Filho (1971) assure the stored cells, when recently harvested, were chemically and biologically normal. A complete autopsy was performed on all animals; a fragment of every organ or tissue (with special attention to the digestive tract, liver, pancreas, testis and

lymphoid tissue) was fixed in buffered 10% formalin. Sections were prepared by routine histological methods and stained with hematoxylin and eosin.

#### RESULTS

##### Chemical analysis

Moisture, 5%; total nitrogen (dry matter), 7.1%; ribonucleic acid (dry matter), 12%; amino acids (mg per g of nitrogen), lysine:498, histidine:115, arginine:249, cystine:36, aspartic acid:546, threonine:304, serine:278, glutamic acid:765, proline:66, glycine:266, alanine:308, valine:310, methionine:undetected, isoleucine:266, leucine:438, tyrosine:178, phenylalanine:232, and tryptophan:75. Limiting factor: sulphur amino acids and E/T (which indicates, according to FAO/WHO (1965), the content of the essential amino acid nitrogen expressed in grams of essential amino acids per gram of total amino acid nitrogen): 2.11.

##### Growth assay

Results are summarized in Figure 1. Mean weight gain by rat daily: 1.06 ± 0.14g (mean ± S.E.); mean protein intake by rat daily: 0.90 ± 0.12g (mean ± S.E.).

The postmortem examinations have not shown any morphological alterations which should be attributed to the presence of toxic compounds. Except for irregular and bristling hair, no gross macroscopic abnormalities were seen. Histologically, the only significant changes were in the testis, represented by degenerative alterations in the germinative epithelium; some rats even showed a complete absence of spermatozooids and spermatids and a characteristic picture of multi-nucleated giant-cells formation was observed; in some cases, it looked as if the number of Sertoli cells increased; a complete atrophy of tubules was not observed. The rats from the control group did not show any morphological or histological abnormalities.

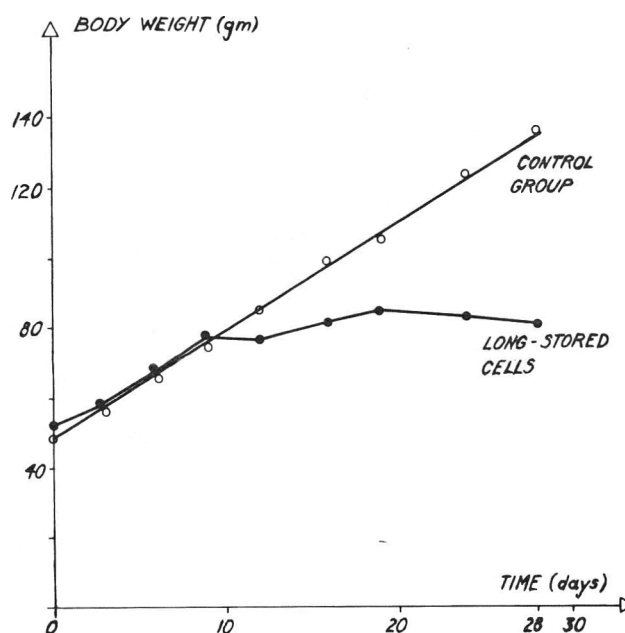


Fig. 1—Growth curves of rats fed the sample and control diets.

Nondetection of methionine was the sole abnormal data from the chemical analysis. However, the biological value observed for the diet which contained 6.25% casein and 11.5% long-stored cells was surprisingly low. In spite of a 0.2% DL-methionine addition, the body weight increases were much lower than those reported by Elias and Bressani (1970) for a diet containing only 5% casein. Had the decrease in biological value reached only the levels secured by 5% casein, we could affirm that the cells have "no significant biological value" and do not contribute to the protein content of our assay (10% on a crude protein basis). However, a drop below this level of efficiency suggests that the old cells have a negative effect upon the utilization of the total mixture.

#### Histo-pathological examinations

The histo-pathological examinations did not reveal the presence of any toxic

effect generated during the 28 days of our experiment. The delay in growth under a diet with optimum concentrations of the nonprotein ingredients, is suggestive exclusively of a protein deficiency and does not imply any toxic effect. Even the changes observed in the testis may be explained by a protein malnutrition, according to Mason (1926) who studied these effects, as in our case, in fast growing rats.

It is possible that old cells had a negative effect upon assimilative functions. Indeed, after 14 days of assay, when the initial growth rate decreased, stools of a semi-solid consistency were observed. This in itself certainly could impair total assimilation of the diet and hinder even the correct utilization of perfectly balanced mixtures.

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