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# LIQUIFYING COD FISH FRAMES UNDER ACIDIC CONDITIONS WITH A FUNGAL ENZYME

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## ABSTRACT

*An acidic protease (Newlase A from* Rhizopus niveus, *Amano) was evaluated as to its ability to reduce viscosity of ground cod fish frames without excessive hydrolysis. Newlase A had an optimal pH of 3.5 and an optimal temperature of 60C with Azocoll as substrate. The enzyme was more stable in the ground fish mixture at pH 3.5 than it was in citrate:phosphate buffer of the same pH. The enzyme hydrolyzed the fish proteins more extensively and decreased the viscosity of the ground fish more between 40–50C over an extended time period than at its optimal temperature of 60C. It is likely that the lower optimal temperature under these conditions is related to its greater stability at the lower temperatures. The energy of activation of the deactivation of the enzyme was 44.5 Kcal per mol-degree. Over a period of 75 min at 45C there was a decrease in viscosity of the ground fish with added enzyme of some two orders of magnitude. After a 3-h incubation, total soluble protein increased from 30% to 48%; the amount of each molecular size fraction produced decreased in the following order: 1–3, >10, <1, and 3–10 kdaltons.*

## INTRODUCTION

Disposal of fishery by-products has become a major concern for the U.S. fishing industry. Traditionally, much of this material has been converted to powdered fish meal by a combined process of cooking, separation of solubles from insolubles, concentration of the solubles and dehydration of the insolubles.

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Many former plants, however, have not been able to maintain operation due to an inability to meet new environmental regulations. At times, this problem has been sufficiently serious to close off a fishery for which there is a good market for the primary product.

To provide data to overcome this problem, we began studying requirements for converting fish by-products to a liquid fertilizer. It was desired to have a process that had low capitalization costs and would be simple to operate. A low pH is generally needed in this type of product to prevent microbial spoilage. There is an intense interest in the use of organic fertilizers, not only from the point of view of producing "organic" produce, but also because its use results in much less run-off of nitrogen and contamination of ground water and oceans or lakes. The leaching problem of a fertilizer in the soil would be reduced if the proteins could be maintained at a relatively high molecular weight; on the other hand, to distribute a liquid fish fertilizer a low viscosity would make it easier to pump, spray or dehydrate. Thus, an enzyme that would significantly reduce viscosity while not giving an excessive amount of low molecular weight nitrogen compounds would be ideal. It would also be desirable to terminate the enzymic reaction by heating.

An initial survey of several acidic proteases indicated that a preparation from a mold, *Rhizopus niveus* (a product of Amano International Enzyme Company) looked promising from both effectiveness and cost. The following presents the results of a study evaluating this enzyme for the process and product described above.

## MATERIALS AND METHODS

### Materials

Atlantic cod (*Gadus morhua*) frames consisting of the head and skeleton with attached flesh remaining after filleting were obtained fresh from a local fish processor in Gloucester, Massachusetts and transported to the laboratory on ice. Cod is generally gutted at sea and filleted on shore. These cod frames constitute an important by-product in the Northeast part of the United States. Cod frames also provided a system that is not as complex as that which would include the viscera or waste from high fat-containing fish. Frames were ground through a 0.64 cm mesh meat grinder (Hobart model 4822). We used phosphoric acid to achieve the acid pH required, since it was also desired to adjust the N-P ratio in the product.

Newlase A from *R. niveus* was obtained from Amano International Enzyme Co., Inc., Troy, VA. Bovine serum albumin, casein, citric acid, trichloracetic acid (TCA), 2,4,6-trinitrobenzenesulfonic acid (TNBS) and Azocoll were

obtained from Sigma Chemical Co., St. Louis, MO. Azocoll is a dye-impregnated hide powder used as a nonspecific protease substrate. Cupric sulfate was a product from Mallinckrodt Chemical Works, St. Louis, MO. All other reagents were obtained from Fisher Scientific Co., Pittsburgh, PA.

## Methods

**Assay of Newlase A Activity**. The Newlase A preparation had an activity of 6250 Azocoll units per g where an Azocoll unit is defined as the activity equal to a change in $A_{520}$ of 0.1 per 15 min at pH 3.5 and 37C. It was found that different batches of Azocoll gave different activities with the same enzyme preparation. Thus, cross-checking of different enzyme preparations should be done using the same batch of Azocoll. The method using Azocoll was similar to that of Dean and Domnas (1983). Enzyme was added to buffer at the appropriate pH to obtain a total volume of 5.0 ml. Values of pH of 5 or less were achieved by the appropriate mixtures of 0.1M citric acid and 0.1M $Na_2HPO_4$ while pH values at 6 and above were achieved with 0.1M sodium phosphate. After temperature equilibration and addition of enzyme, the reaction was initiated with the addition of 50 mg Azocoll. After 15 min, the suspension was filtered and its absorbance was measured at 520 nm. Absorbances without enzyme were subtracted and were generally less than 0.05 absorbance units. Assay of Newlase A with Azocoll as substrate was used to determine the kinetic characteristics of the enzyme.

Activity of Newlase A in the ground fish tissue was estimated by measuring the increase of soluble amino groups. Ground fish was heated to the temperature of assay. A sample was taken immediately for the initial reading. Thereafter, two samples (0.02% Newlase A had been added to one) were incubated at the appropriate temperature. At various time intervals 10 g samples of both control (without enzyme) and enzyme-treated ground fish were taken, blended with 10 ml of 10% TCA, and filtered through qualitative Whatman No. 1 filter paper. A 0.10 ml amount of this extract was then diluted with an appropriate amount of 10% TCA (usually 0.9 ml), and 0.05 ml was used for the determination of free amino groups with 2,4,6-trinitrobenzenesulfonic acid by the method of Snyder and Sobocinski (1975). Glycine was used to create a standard curve.

**Stability Measurements**. Stability of Newlase A was determined by heating 20 ml of the appropriate buffer (see above) to the desired temperature after which 5 ml of 2% enzyme solution was added. At appropriate intervals duplicate samples of 0.11 ml were diluted to 5 ml with the citrate-phosphate buffer at pH 3.5. The remaining activity was then determined under the standard conditions using 50 mg of Azocoll for 15 min at 37C.

Enzyme stability was also determined in the ground fish. A 200 g amount of ground fish frames was adjusted to pH 3.5 with 85% phosphoric acid. The acidified ground fish was then brought to the appropriate temperature. Two grams of Newlase A was then added, and triplicate samples of 0.5 g were removed and added to 4.5 ml of pH 3.5 buffer. Fifty mg of Azocoll was added to initiate the reaction; after 15 min at 37C, the suspension was filtered and the absorbance read at 520 nm. Controls containing only Azocoll or fish plus Azocoll with no added enzyme were subtracted from the readings of the samples containing the enzyme.

**Molecular Size Distribution of Reaction Products from Fish.** A 100 g portion of ground fish tissue was centrifuged at 4C in a No. 35 rotor of a Beckman model L5-65B ultracentrifuge at 29,000 rpm for 15 min. The supernatant fraction provided the soluble fraction of the unheated sample. Other ground fish was separated into 2 1-kg samples and heated to 45C after adjusting the pH to 3.5 with 85% phosphoric acid. After 45C was reached a 100 g sample was taken to represent 0 time. This and all subsequent samples were centrifuged as described above. Then, 0.02% of Newlase A was added to one of the heated samples. At various time intervals approximately 100 g of both a control sample without enzyme and a sample containing enzyme were removed for examination. Each soluble extract was transferred in 20 ml amounts to a Diaflo ultrafiltration unit where it was filtered at 5C using 40 psi obtained with nitrogen. Other portions of the supernatant fraction from the centrifugation were placed into either 10,000 or 3,000 molecular weight centricon units and centrifuged at 5C at maximum speed in an IEC model cl table top centrifuge. Nitrogen content in each of the filtrates was determined by Kjeldahl (Kjeltec manual, Perstorp Analytical Inc., Herndon, VA).

**Analytical Procedures.** Protein content of minced fish was determined by the biuret method of Torten and Whitaker (1963). Moisture was determined after drying at 102C for 18 h (AOAC 1980).

Viscosity was measured with a Brookfield dial reading viscometer with helipath stand and T-bar spindles. Readings were converted to centipoise units by the appropriate conversion factor corresponding to each viscometer spindle and speed combination. Viscosity changes in the ground fish with or without added enzyme were measured on the same samples used to determine extent of hydrolysis. Measurements of extent of hydrolysis were done first followed by viscosity measurements. Thus, data on viscosity changes lagged hydrolysis data by 15 min.

**Statistical Analysis.** Analysis of variance was performed according to Steel and Torrie (1960) at the 95% confidence level.

## RESULTS

Evaluation of the long term stability of the ground fish towards microbial spoilage showed that a pH of 3.5 was required to achieve a shelf-life of 12 months when phosphoric acid was used to lower the pH (Kelleher, unpublished results); thus, this pH was chosen as the most desirable for processing the ground fish. After a preliminary survey evaluating kinetic properties of some commercially available proteases with reported activities in the acid pH range and taking into consideration the cost of the enzyme, Newlase A (Amano International Co., Inc., Troy, VA) was chosen for examination in detail. Azocoll was chosen as the substrate for preliminary screening due to the simplicity of the assay; similar results qualitatively were obtained when casein was used as substrate instead of Azocoll.

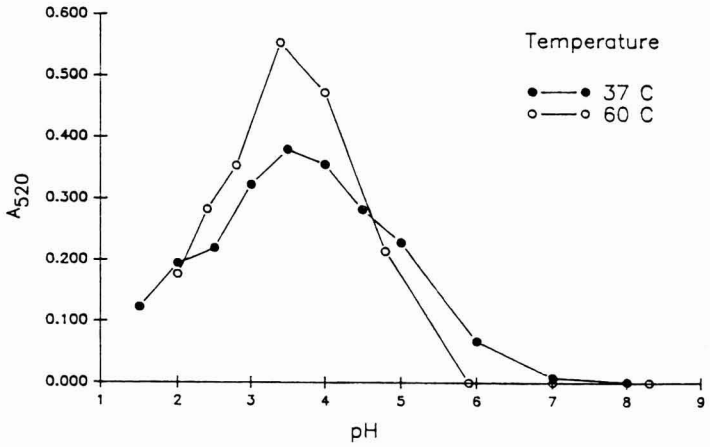


FIG. 1. ACTIVITY OF NEWLASE A AS A FUNCTION OF pH WITH AZOCOLL AS SUBSTRATE AT 37C AND 60C

At either 37C or 60C the optimal pH of Newlase A with Azocoll as substrate was approximately 3.5 after an incubation time of 15 min (Fig. 1). The decrease in activity as a function of pH on either side of the optimum was somewhat greater for the enzyme at 60C compared to 37C. This is likely due to the greater instability of the enzyme at the higher temperature. At pH 3.5 Newlase A showed an optimal temperature of 60C with Azocoll as substrate (Fig. 2). The activation energy of the reaction over the range from 20 to 60C was 18.5 Kcal per mol degree.

Hydrolysis of the proteins of the ground fish frames was followed by measuring the appearance of free amino groups. Figure 3 shows the time

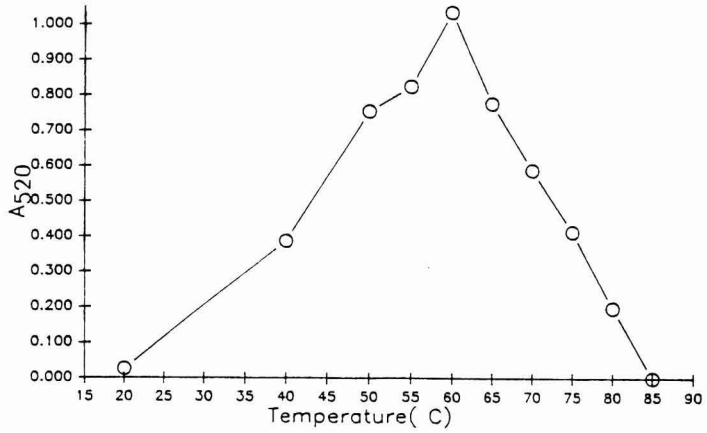


FIG. 2. ACTIVITY OF NEWLASE A AS A FUNCTION OF TEMPERATURE WITH AZOCOLL AS SUBSTRATE AT pH 3.5

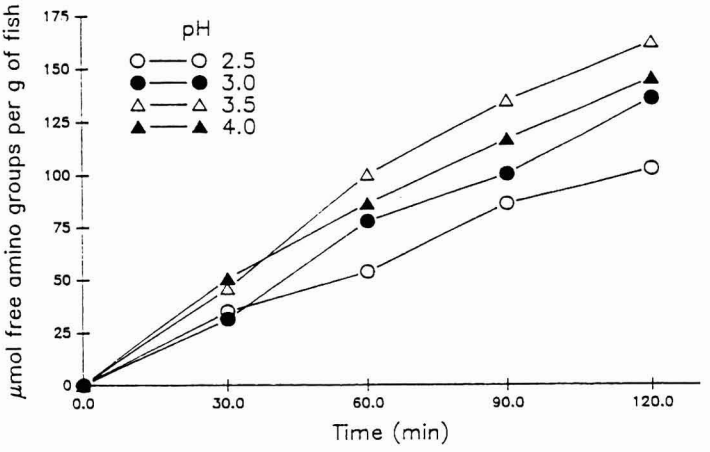


FIG. 3. PRODUCTION OF FREE AMINO GROUPS WITH TIME FROM GROUND COD FRAMES OVER A pH RANGE FROM 2.5 TO 4.0 AT 40C

dependence of the production of free amino groups at pH values from 2.5 to 4.0 at 40C. Activity was maximal at pH 3.5 at times of 60 min or greater; the amount of free amino groups was similar at values of pH of 3.5 and 4.0 after 30 min. The time course of free amino production in the ground fish frames by Newlase A as a function of temperature at pH 3.5 is shown in Fig. 4. After 2 h of incubation, activity was greatest over the range of 40–50C with 45C giving

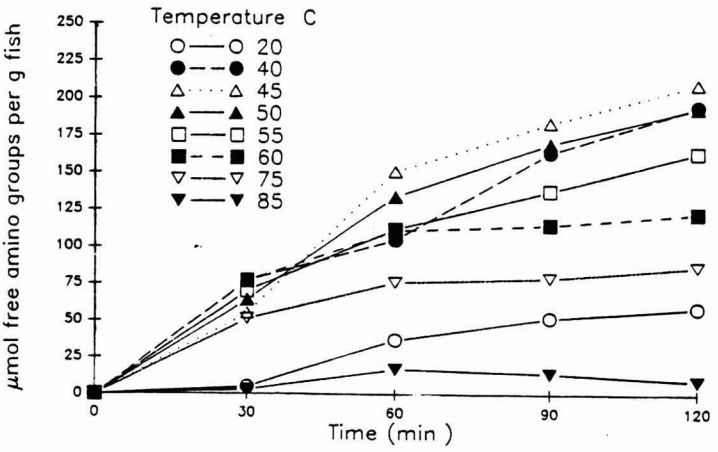


FIG. 4. PRODUCTION OF FREE AMINO GROUPS WITH TIME FROM GROUND COD FRAMES OVER A TEMPERATURE RANGE OF 20C TO 85C AT pH 3.5

slightly more activity than 40 and 50C. The relative activity of Newlase A at the different temperatures is dependent on the reaction time. After 30 min of reaction, somewhat greater production of free amino groups is observed between 50 to 60C. Likely, these results are a reflection of the hydrolytic effect of the enzyme that increases with increasing temperature while at the same time enzymic activity is lost by denaturation or proteolysis. Thus, with times greater than 60 min the amount of free amino groups produced at 60C is less than that produced at 55C which in turn is less than that produced over the range of 40–50C. Low productivity at 85C would indicate rapid deactivation of the enzyme; the slight production observed might be due to nonenzymic hydrolysis. At 20C (approximately room temperature), Newlase A was considerably less active than it was at the higher temperatures.

The primary purpose of treating the ground cod frames with a proteolytic enzyme was to reduce the viscosity to make the material easier to pump, particularly after concentration. The effect of adding 0.02% of a Newlase A preparation on change in viscosity of the ground cod frames over a period of 1 h 45 min at temperatures ranging from 20 to 85C is shown in Fig. 5. Included in Fig. 5 are the viscosities of heated ground fish frames to which no enzyme was added. Viscosity measurements were made at the temperature at which the samples were incubated. This was done because of the need to obtain readings on several samples quickly to avoid changes during the period of temperature adjustment. Thus, absolute readings of viscosity can only be compared approximately. The extent of the reduction, however, within a sample at any one temperature and any cessation of reduction can be observed. One consequence

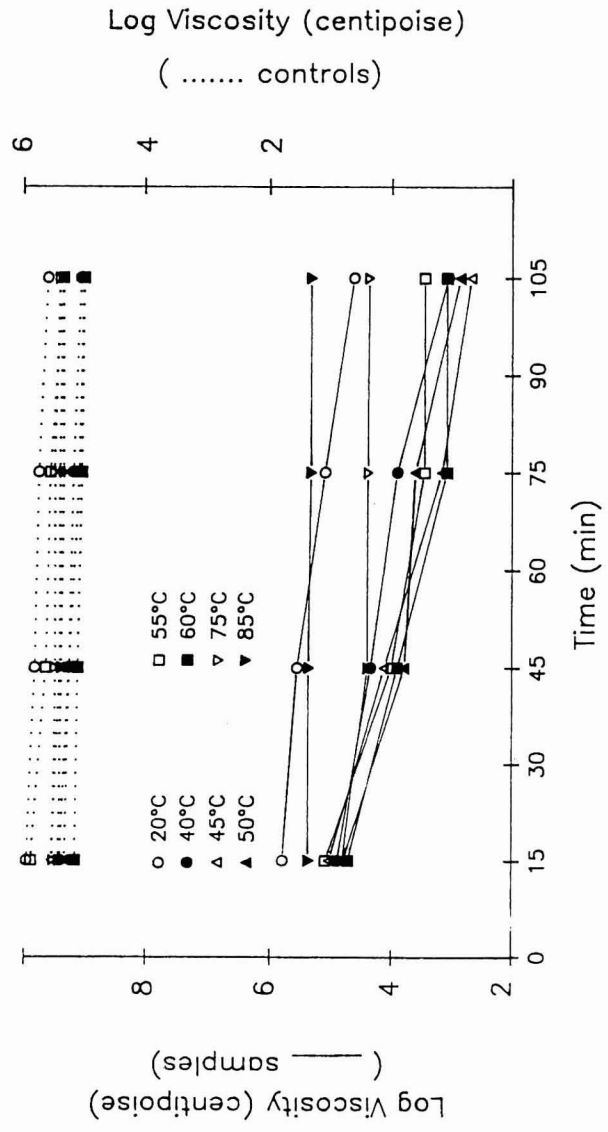


FIG. 5. CHANGES IN VISCOSITY/CONSISTENCY OF GROUND COD FRAMES ON TREATMENT WITH NEWLASE A OVER A TEMPERATURE RANGE OF 20C TO 85C
Viscosity measurements were made at the temperatures of incubation.
The top set of curves were changes observed without added enzyme.

of measuring the viscosities at the individual incubation temperatures is that the initial values are not constant. Some of the variation in the initial values may be a reflection of a temperature effect on the viscosity and some may be related to the difficulty in obtaining uniform samples of such a heterogenous substance. In Fig. 5 viscometer readings are represented. It is, however, likely that values approaching true viscosities were only reached at 10,000 centipoise or less. The initial material contained much particulate matter and was not uniform. Nevertheless, the instrument value gives an indication of the "consistency" of the material.

In general, the changes in viscosity observed was what might be expected for an enzyme-catalyzed reaction. Significant reduction in viscosity was achieved at most all temperatures with somewhat increasing rates as the temperature was increased. There was, however, no change in viscosity with the sample at 85C. This is likely due to inactivation of the enzyme. The decrease in viscosity was lower at 75C than it was between 40 to 60C. This probably is caused by early deactivation of the enzyme. Enzymic treatment reduced viscosity at 55C and 60C for up to 1 h; thereafter there was no change. Again, deactivation of the enzyme is a reasonable explanation for loss of the ability of the Newlase A to reduce viscosity after this time period. At 45C viscosity was decreased by two orders of magnitude.

The effect of temperature on the production of soluble free amino groups and the reduction in viscosity of the ground cod frames indicated that stability of Newlase A at pH 3.5 would be an important factor in utilizing this enzyme to hydrolyze the proteins of cod fish frames. The activity of Newlase A was determined with Azocoll after incubation of the enzyme in either citrate-phosphate buffer or ground fish tissue at pH 3.5 at various temperatures. Typical results are shown for three different temperatures (Fig. 6). At 90C the rate of deactivation of the enzyme in buffer was too fast to be determined by our methodology. The enzyme displayed greater stability in ground fish than in buffer. This indicates that Newlase A may interact with some component of the ground fish that increases its stabilization. Alternatively, the results indicate that the enzyme may undergo autolysis which can be reduced when there is a large amount of other proteins for it to attack. Since Newlase A is more stable in the ground fish than buffer, it seems unlikely that it is destroyed to any great extent by proteolytic enzymes present in the fish tissue. An Arrhenius plot of the first order deactivation rate constants of Newlase A in citrate-phosphate buffer give an energy of activation of deactivation of 44.5 Kcal per mol-degree (Fig. 7). Deactivation rate constants observed in ground fish at two temperatures are included for comparison; these data were not included in the calculation.

Changes in total soluble nitrogen were determined for ground cod fish frames treated with Newlase A at pH 3.5 and 45C (Table 1). The soluble nitrogen fraction was separated into fractions by molecular size, i.e., $>$ 10,

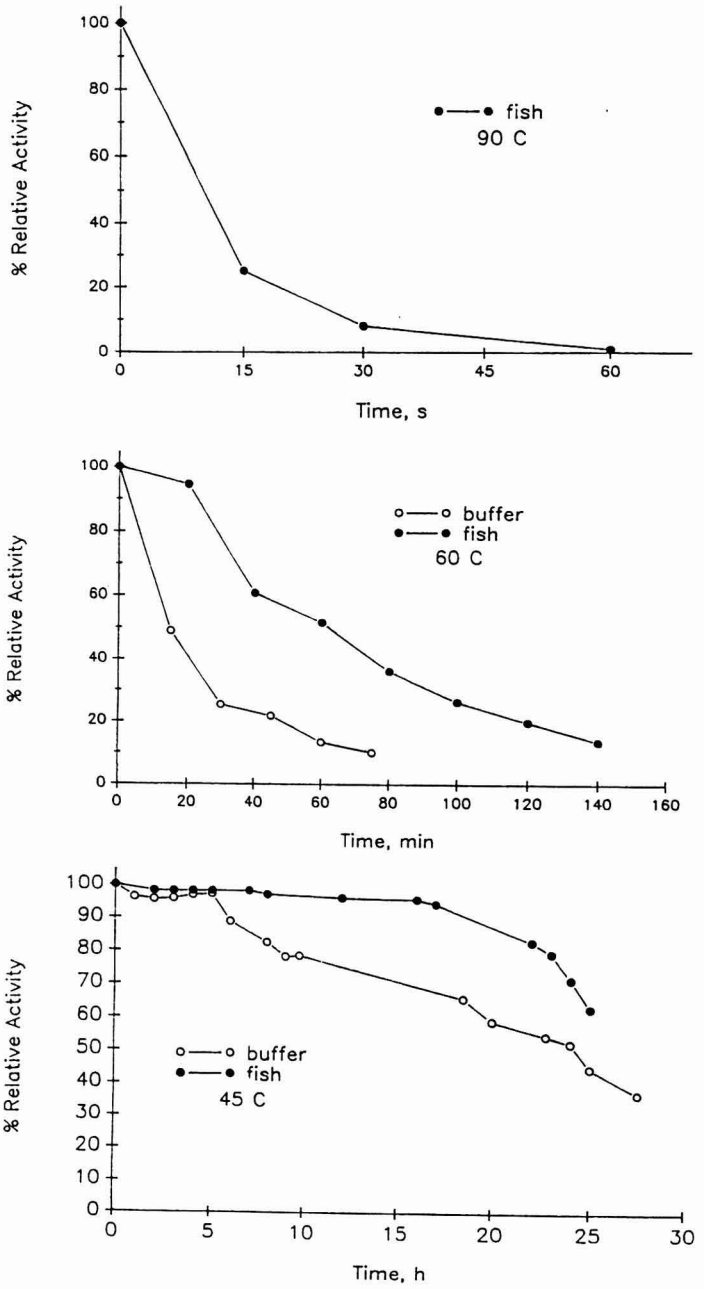


FIG. 6. THERMAL STABILITY OF NEWLASE A IN BUFFER (○) AND IN GROUND COD (●) AFTER INCUBATION FOR THE INDICATED TIMES AT 90C (top), 60C (center), AND 45C (bottom)
The experiments were carried out as described in Materials and Methods

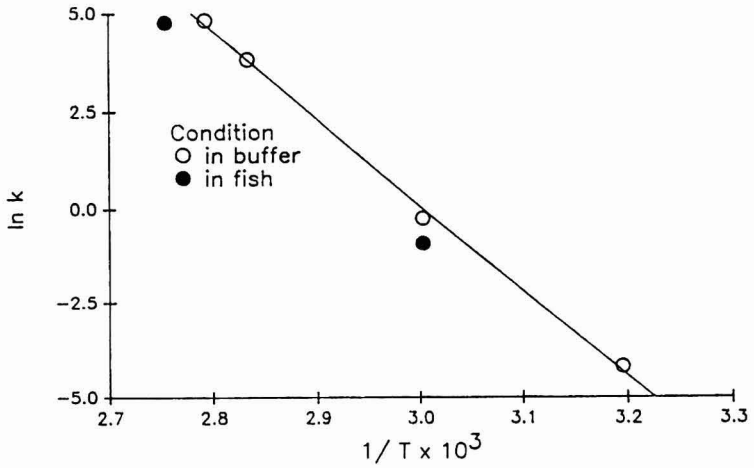


FIG. 7. ARRHENIUS PLOT OF THE FIRST ORDER DEACTIVATION RATE CONSTANTS OF NEWLASE A IN CITRATE-PHOSPHATE BUFFER (○) AND IN GROUND COD FRAMES (●) The activation energy of 44.5 Kcal per mol-degree was calculated from data obtained using buffer.

TABLE 1.
CHANGE WITH TIME OF SOLUBLE NITROGEN FRACTIONS OF GROUND COD FRAMES ON TREATMENT WITH NEWLASE A AT pH 3.5 AND 45 C

| | | Individual fractions | | | |
|---|---|---|---|---|---|
| Time (min) | Total soluble N | >10 Kdal | 3-10 Kdal | 1-3 Kdal | <1 Kdal |
| 0 (unheated) | 30.2 | 20.5 | 0 | 3.2 | 6.5 |
| 0 | 32.7 | 21.4 | 1.6 | 3.2 | 6.5 |
| 30 | 40.0 | 15.0 | 6.0 | 11.7 | 7.3 |
| 60 | 42.3 | 16.5 | 5.6 | 12.1 | 8.1 |
| 120 | 46.0 | 15.8 | 5.2 | 14.5 | 10.5 |
| 180 | 48.0 | 14.5 | 4.8 | 18.2 | 10.5 |

Data are expressed as per cent of total N. Total N of the ground fish equalled 24.8 mg N per g wet weight of fish (moisture content of 81%).

3–10, 1–3, and < 1 kdaltons. There was a small increase in soluble nitrogen as the temperature of the sample was raised from 4C to the incubation temperature of 45C. This was followed by an increase of over 7% in the first 30 min and smaller increases for the next 150 min. The soluble fraction with molecular weight > 10 kdaltons decreased rapidly in the first 30 min and then remained relatively stable over the next 2.5 h. There was a rapid increase in the 3–10

kdalton fraction followed by a very slow decrease. The soluble fraction of molecular weight 1–3 kdaltons increased rapidly in the first 30 min and more slowly but steadily for the next 2.5 h, while the fraction of $<1$ kdalton increased slowly for the first 2 h and no change was observed thereafter. After 3 h incubation the amount of soluble nitrogen was greatest in the 1–3 kdalton molecular weight fraction, followed by the fraction with molecular weight $>10$ kdaltons. The fraction with molecular weight below 1 kdalton was next while soluble nitrogen of molecular weight 3–10 kdaltons represented the smallest fraction. The corresponding percentages of these fractions was 37.9%, 30.2%, 21.9% and 10.0% of the total soluble nitrogen. That over 50% of the nitrogen remained insoluble and that net changes in the fraction with molecular weight $>10$ kdaltons did not change much after the first 30 min indicates that the specificity of Newlase A against the proteins of the ground cod fish frames was limited.

## DISCUSSION

Although proteases operating at alkaline pH have often been used for hydrolysis of fishery by-products (Jacobsen and Lykke-Rasumssen 1984; Schaffeld *et al.* 1989), the stabilizing effect towards microbial spoilage of a low pH makes it attractive to reach that pH as early in the processing as possible. This will be particularly true if an automatic continuous process is to be used that might involve pumping the material through the various process stages. A breakdown of machinery and a hold-up of the material could be a problem if that material were at neutral pH. Greater flexibility in cleaning schedules would be another advantage of a material at low pH since the acid pH would prevent rapid microbial growth in the material. Early treatment with acid would be particularly useful with fish that were aged postmortem longer than desired, since they could contain high microbial counts and volatile nitrogen (del Valle and Aguilera 1991); acidification of volatile nitrogen bases would both alleviate a potential odor problem and produce higher yields of nitrogen.

A pH of 3.5 was chosen, since we found that this pH was required to give long term stability (at least 1 year) to the product. Other acids such as formic or propionic may be effective at higher pH values (Haard *et al.* 1985). We added phosphoric acid because it was desired to balance the nitrogen-phosphorus ratio of the material for use as a fertilizer. Phosphoric acid has little buffering capacity between 3.5 and 4 with $pK_a$ values of 2.1 and 7.2 (Dziezak 1990). During enzymic treatment of the ground fish frames at pH 3.5, it was necessary to add acid to maintain that pH. The amount necessary was initially high and decreased with time. The consumption of acid was due both to hydrolysis of the protein and demineralization of the bone (Kelleher, unpublished results).

Newlase A met the requirements for processing fish frames in an acid environment well. It had good activity and stability at pH 3.5. Its reasonably broad activity around pH 3.5 would allow for some flexibility of pH maintenance. This could be particularly important since acidification of the product is necessary to maintain the desired pH. The enzyme did not appear to be particularly sensitive to temperature and gave reasonable performance over a fairly broad temperature range, both with respect to production of free amino groups and decrease in viscosity. At the same time the enzyme could be deactivated quite readily at temperatures of 85–90C if it became of interest to control the amount of hydrolysis in the product. There appeared to be rather limited specificity of activity of the enzyme and thus no excessive protein breakdown. This may not be suitable for some products, but it is likely an advantage in production of both fertilizer and animal feed where maintenance of size of the peptides may be useful. In the case of fertilizer this might provide less wash out and slower release of the nitrogen, while with animal feeds it might control the production of bitter compounds by reducing the amount of low molecular weight peptides and make recovery of protein easier.

In the case of the product that we were interested in producing, i.e., a fertilizer for dispersal in liquid form, it was desired to lower the viscosity as much as feasible without excessive breakdown of the proteins. The Newlase A enzyme did this well, since there was a reduction of viscosity (or consistency) of greater than two orders of magnitude at 45C and almost as much at 50C. This was accomplished with an estimated hydrolysis of the proteins of only 29% at 45C. This estimate was based on measuring free amino groups and assuming an average molecular weight of 130 for the amino acids and that all of the hydrolyzed groups became soluble. Sugiyama *et al.* (1991) treated defatted sardine meal with a number of proteases including "Newlase" for the preparation of "nutritious fish protein hydrolysates" for human consumption. They found the enzyme produced major fractions with molecular weights centered around 1.5 and 6.5 kdaltons.

Although short term studies *in vitro* indicated that Newlase A had an optimal temperature of approximately 60C, studies of the effectiveness of the enzyme in the ground fish frames over longer time periods generally showed that optimal results were achieved over the temperature range 40–50C with indication that 45C might be the maximum. Although Newlase A was more stable in ground fish than it was in citrate-phosphate buffer, it nevertheless seems likely that this shift in temperature optimum is due to deactivation of the enzyme at the higher temperature. For example, the enzyme in ground fish lost approximately 50% of its activity at 60C with 60 min incubation time at pH 3.5. The activation energy of 44.5 Kcal per mol indicates that the deactivation was highly dependent on temperature, and therefore the deactivation would increase rapidly above 60C. To optimize any process with respect to temperature at pH

3.5, it would be necessary to know the amount of hydrolysis that was desired. If a relatively low amount of hydrolysis is needed, a temperature of 60C might provide this more quickly than a lower temperature, e.g., 45C, for a given amount of enzyme. On the other hand, to obtain a higher degree of hydrolysis, it may be necessary to operate at the lower temperature to avoid the more rapid deactivation of the enzyme at the higher temperature.

Although the conditions reported in this paper were developed for a product in which it was desired to reduce viscosity, minimize hydrolysis and add phosphate, it is likely that Newlase A might be a useful catalyst in any situation where a pH between 3 and 4 was to be used, and where it was desired to reduce the viscosity without excessive hydrolysis.

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# SOME PROPERTIES OF FISH GELS MADE FROM SEVERAL NORTHWEST ATLANTIC SPECIES IN THE PRESENCE OF HIGH AND LOW SALT

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## ABSTRACT

*The relative effect of NaCl on true strain and stress at failure varied considerably among unfrozen fish species. Values of true strain were always greater in samples with 3% salt in the absence of cryoprotectants. The effect of NaCl on stress varied more widely than on true strain; in a couple of cases, stress at failure was higher for fish gels without 3% sodium chloride. Three of four fatty species (Atlantic mackerel, Atlantic menhaden and bluefish) were among the samples that had the lowest values of both true strain and stress. For red hake, seasonal effects of salt on gelation properties were observed. True strain and stress values of red hake were higher without added salt than they were with 3% added salt when cryoprotectants were added to the samples and the samples were stored for one week at −20C.*

## INTRODUCTION

The generally accepted hypothesis explaining the formation of fish gels has been that a high concentration of NaCl is required to solubilize the myofibrillar proteins (Suzuki 1981; Lee 1984, 1986; Shimizu 1985), particularly myosin and actomyosin, which can then gel upon heating as the proteins denature, interact and aggregate (Hermansson *et al.* 1986; Wicker *et al.* 1989; Niwa *et al.* 1989;

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Xiong and Brekke 1990). With many people interested in reducing sodium intake (Wirth 1990), we evaluated the requirement of salt for gel formation and observed that good fish gels formed without the addition of the 2–3% (0.34–0.51 M) NaCl thought to be required (Hennigar *et al.* 1988, 1989; Vareltzis *et al.* 1989). These "low-salt" gels contained approximately 0.15% (26 mM) NaCl, a level of salt used to aid in the dewatering process.

The quality of fish gels prepared without added salt compared to gels with 2.5–3% salt as measured by the fold test was species dependent. The purpose of the experiments reported here was to evaluate a large number of species from the Northwest Atlantic to determine the properties of their gels formed at low salt concentration. Factors such as seasonality and the effect of cryoprotectants on gel formation were also evaluated.

## MATERIALS AND METHODS

### Fish Species Used

Fish were purchased from day boats fishing out of Gloucester, MA. The fish were transported on ice to the laboratory where they were held overnight in a refrigerator at 4C (still on ice) prior to processing. Listed in Table 1, 15 species of fish were examined. In addition, the effect of low and high salt concentrations on the gelation properties of a commercially prepared Alaska pollack surimi (grade A) was evaluated.

### Preparation of Washed, Minced Fish

Fish were filleted and skinned by hand after which the fillets were ground through a 0.64 cm plate in a Hobart grinder, Model 4822 (Troy, OH). Minced fish was mixed with 3 volumes of chilled, deionized, distilled water. The mixture was stirred for 2 min and then allowed to settle for 15 min. The washed mince was strained through a fiberglass screen (0.3 mm). This washing process was repeated two more times. The last wash always contained 0.15% NaCl. The washed mince was placed in the center of a bed of cheesecloth 4 layers thick (0.6 × 1.2 m). The cheesecloth was then folded over the mince to form a secure package to prevent loss of mince when pressure was applied. The pressure was applied by a hydraulic, hand-operated Carver laboratory press (Summit, NJ) by increasing the pressure gradually for 2 min until a gauge pressure of 10,000 psig was reached. The material was held at that pressure for 15 min. The dewatered mince was removed from the press and immediately processed into gels.

Most gels were prepared in the absence of cryoprotectants; with some samples of red hake, a mixture of 4% sucrose, 4% sorbitol and 0.2% sodium tripolyphosphate (STPP) based on the weight of the washed mince was used. When cryoprotectants were used, the samples were stored for 1 week at −20C before gel preparation.

## Gel Preparation

Two different gels were prepared from each batch of minced fish: one with an additional 3% NaCl added and one without added NaCl. A 200 g portion of washed mince was placed in a food processor (Robot-Coupe R301 Ultra). The dewatered mince at a temperature of 5C or below was placed in a bowl that had been previously cooled to −20C, and the mince was chopped at high speed for 2 min. In the samples to which NaCl was added, this was done slowly during the first minute of chopping. The temperature of the fish paste was kept below 15C at all times. The fish paste was stuffed into stainless steel tubes (i.d. = 1.87 cm, length = 17.75 cm) with minimum incorporation of air bubbles and sealed at both ends. The tubes were placed in a 40C water bath for 30 min and in a 90C water bath for 20 min. Immediately after heating the tubes were cooled in a mixture of ice and water for 10 min. Gels were held refrigerated in sealed plastic bags for between 24–48 h until tested.

## Measurement of Strain and Stress at Failure

Gels were warmed to room temperature, cut into 10 cm lengths and milled to form dumbbells 28.7 mm long with a minimum diameter of 1 mm as described by Montejano *et al.* (1983). The gels were then subjected to torsion analysis as described by Wu *et al.* (1985). The torsion device was attached to a Brookfield viscometer Model DV II (Stoughton, MA). Stress and strain at failure were calculated from the data using the equations given by Hamann (1983).

## Other Tests

The fold test was applied to 3 mm thick slices of the gels according to the procedure of Kudo *et al.* (1973). A score of 5 was a gel slice that could be folded into quarters without cracking. A score of 4 was for a gel that could be folded into half without cracking, a score of 3 indicated some cracking when folded in half, and a score of 2 indicated that the gel broke on folding.

The pH of either the minced fish or gel was determined by blending 10 g of sample for 1 min with 90 ml of distilled water and determining the pH with a glass electrode and pH meter. The moisture content of the minced fish or gels

was determined as the mean moisture loss after drying in an oven at 110C for at least 18 h with duplicate samples. Values of pH and moisture are reported for the starting material and the washed, dewatered mince.

### Statistical Analysis

Statistical analysis of means was performed using one way or two way analysis of variance as described by Ryan *et al.* (1985). Multiple comparison of means was determined using the procedures described by Steel and Torrie (1960).

## RESULTS AND DISCUSSION

### Survey of Species

Values of true strain, stress and fold-test scores are given for the gels prepared from 15 Northwest Atlantic species, using unfrozen tissue without added salt and with added salt (Table 1). The samples without added salt had been dewatered in the presence of 0.15% NaCl and thus the gels contained approximately this concentration. The samples with added salt contained 3% salt plus the 0.15% used in dewatering. The percent water and pH of both the original fish tissue and the washed, dewatered mince are also presented in the table. With two species of dark fleshed fish, Atlantic mackerel and bluefish, gels prepared from whole muscle and from the light or ordinary muscle were examined. Also included in the table are gels prepared with and without added salt from commercial Alaska pollack surimi. The percent water and pH for this sample refers to the thawed surimi; this is the only sample in this table to which cryoprotectants had been added. Cryoprotectants were generally not added to the gels prepared from other fish species, since the purpose of the experiments was to evaluate salt content on gel formation *per se* and not to duplicate commercial procedures. In all cases, gels were prepared without freezing the samples; thus, cryoprotectants were not required to stabilize the proteins.

In all cases, comparisons of properties between the species reported in Table 1 in the presence and absence of added salt, i.e., at high and low salt contents, were performed on gels made from the same batch of washed, minced fish. The only difference between the samples with and without salt was the incorporation of 3% salt during the mixing procedure. Samples without added salt were mixed in the same way for the same time but did not have salt added to them.

True strain at failure has been reported to be a measure of the protein quality of a fish gel (Hamann and Lanier 1987). When the ratio of true stain for gels without and with added salt was calculated, values ranged from 0.47 to 0.91. Five species of fish had low strain ratios with and without added salt.

Three of these were fatty fish, bluefish, menhaden and Atlantic mackerel. When the white muscle was examined separately from the whole muscle, i.e., Atlantic mackerel and bluefish, no improvement was observed. Ocean catfish and cusk were the other two species of fish that had lower ratios of true strain without and with salt than the other species. The other fatty fish examined, Atlantic herring, had a higher ratio (0.75) than bluefish, Atlantic mackerel or menhaden.

Strain is considered a measure of elasticity of the gel. It presumably measures a property similar to the fold test. The three species, cod, dab and red hake, that had high fold test scores in the absence of the high content of NaCl all had relatively high ratios of true strain at low and high salt, but other species that had the same ratio of true strain values had lower fold test scores in the absence of high concentrations of salt. Measurement of strain by a torsion technique is obviously not measuring the same physical properties as those measured by folding. There was, nevertheless, a relation between true strain and fold test scores among all samples with or without salt (correlation coefficient = +0.615). Gels prepared from Alaska pollack surimi also had a high fold test score (5) in the absence of added salt, but the fact that cryoprotectants were present in this product might have affected the results (see section on Effect of Cryoprotectants on True Stress and Strain).

The ratios of stress at failure of the gels prepared with high and low salt of the several species of fish showed an even greater range than did the ratios of true strain. Three of the four species with the lowest ratios (0.48 to 0.69) were the three fatty species that were in the group that had the low values of true strain. It is possible that the low values of true strain and stress for Atlantic mackerel and menhaden was related to the low pH values of these species, which favored denaturation of their muscle proteins.

Another group of six species (including the Alaska pollack surimi) had intermittent ratios of stress with and without salt (0.75 to 0.87), four had essentially a stress ratio of 1, and two, red hake (1.5) and dab (1.2), had ratios $> 1$. Stress is markedly affected by moisture content in surimi-based gels (Hamann and MacDonald 1992). No simple relationship seemed to exist between the moisture content of the washed minces and the absolute values of stress (correlation coefficient = +0.021) or the ratios observed. It seems likely that differences in proteins among the various species were responsible for the differences observed. Variations in properties of surimi gels from various species have been reported (Shimizu *et al.* 1981).

## Seasonal Effects on Red Hake Gels

Seasonal changes were examined for one species, red hake. True strain and stress values with and without added salt are given in Fig. 1 and 2 together with

TABLE 1.
CHARACTERISTICS OF FISH GELS FROM SEVERAL NORTHWEST ATLANTIC SPECIES MADE WITH AND WITHOUT SALT

| Species | Fish | | Mince | | True Strain | Ratio Strain wo/w salt | Stress Kpa | Ratio Stress wo/w salt | Fold Test Score |
|---|---|---|---|---|---|---|---|---|---|
| | %$H_2O$ | pH | %$H_2O$ | pH | | | | | |
| Anarhichadiade | | | | | | | | | |
| **Ocean catfish** (Anarhichas lupus) | | | | | | | | | |
| w salt | 79.9 ± 0.5 | 6.2 | 81.3 ± 0.4 | 6.4 | 1.23 ± 0.12 | 0.61 | 29.9 ± 4.6 | 0.86 | 5 |
| wo salt | 79.9 ± 0.5 | 6.1 | 79.8 ± 0.4 | 6.3 | 0.75 ± 0.12 | | 25.8 ± 1.6 | | 2 |
| Clupeidae | | | | | | | | | |
| **Menhaden** (Brevoortia tyrannus) | | | | | | | | | |
| w salt | 75.5 ± 0.8 | 6.1 | 77.2 ± 0.1 | 6.4 | 1.50 ± 0.11 | 0.51 | 52.7 ± 3.5 | 0.69 | 5 |
| wo salt | 75.5 ± 0.8 | 6.1 | 75.1 ± 0.1 | 6.1 | 0.76 ± 0.05 | | 36.5 ± 7.7 | | 2 |
| **Atlantic herring** (Clupea harengus) | | | | | | | | | |
| w salt | 76.0 ± 0.0 | 6.5 | 76.2 ± 0.4 | 6.3 | 1.32 ± 0.11 | 0.75 | 32.6 ± 1.3 | 0.80 | 5 |
| wo salt | 76.0 ± 0.0 | 6.5 | 78.7 ± 0.5 | 6.7 | 0.99 ± 0.09 | | 26.2 ± 2.6 | | 2.5 |
| Gadidae | | | | | | | | | |
| **Alaska pollock surimi** (Theragra charcogramma) | | | | | | | | | |
| w salt | --- | --- | 73.4 ± 0.5 | 7.3 | 2.16 ± 0.20 | 0.78 | 77.6 ±8.9 | 0.85 | 5 |
| wo salt | --- | --- | 73.4 ± 0.5 | 7.3 | 1.69 ± 0.20 | | 66.2 ± 7.6 | | 5 |

Table 1 (continued)

| Species | Fish | | Mince | | True Strain | Ratio Strain wo/w salt | Stress Kpa | Ratio Stress wo/w salt | Fold Test Score |
|---|---|---|---|---|---|---|---|---|---|
| | %$H_2O$ | pH | %$H_2O$ | pH | | | | | |
| **Atlantic pollock** (Pollachius virens) | | | | | | | | | |
| w salt | 79.8 ± 0.3 | 6.5 | 83.0 ± 0.4 | 6.6 | 1.59 ± 0.08 | 0.74 | 30.8 ± 4.1 | 1.00 | 5 |
| wo salt | 79.8 ± 0.03 | 6.5 | 83.3 ± 0.2 | 6.9 | 1.18 ± 0.09 | | 30.8 ± 3.7 | | 3 |
| **Atlantic cod** (Gadus morhua) | | | | | | | | | |
| w salt | 82.1 ± 0.4 | 6.5 | 83.5 ± 0.1 | 6.7 | 1.99 ± 0.15 | 0.91 | 20.0 ± 2.7 | 0.99 | 5 |
| wo salt | 82.1 ± 0.0 | 6.5 | 84.6 ± 0.2 | 6.8 | 1.82 ± 0.08 | | 19.7 ± 2.2 | | 5 |
| **Cusk** (Brosme brosme) | | | | | | | | | |
| w salt | 80.7 ± 0.5 | 6.5 | 79.2 ± 0.4 | 6.6 | 1.76 ± 0.20 | 0.58 | 33.7 ± 6.5 | 1.02 | 5 |
| wo salt | 80.7 ± 0.5 | 6.5 | 82.2 ± 0.0 | 6.5 | 1.02 ± 0.10 | | 34.4 ± 7.5 | | 3 |
| **Haddock** (Melanogrammus aeglefinus) | | | | | | | | | |
| w salt | 83.2 ± 0.2 | 6.7 | 80.8 ± 0.6 | 6.7 | 1.59 ± 0.08 | 0.85 | 26.1 ± 1.9 | 0.48 | 5 |
| wo salt | 83.2 ± 0.2 | 6.7 | 84.7 ± 0.5 | 7.5 | 1.35 ± 0.05 | | 12.5 ± 1.1 | | 3 |
| **Red hake** (Urophycis chuss) | | | | | | | | | |
| w salt | 82.1 ± 1.9 | 6.9 | 82.3 ± 4.3 | 6.7 | 2.03 ± 0.17 | 0.78 | 18.4 ± 3.0 | 1.54 | 5 |
| wo salt | 82.5 ± 1.5 | 6.9 | 83.4 ± 2.2 | 6.8 | 1.58 ± 0.18 | | 28.4 ± 3.2 | | 4.5 |

Table 1 (continued)

| Species | Fish %$H_2O$ | Fish pH | Mince %$H_2O$ | Mince pH | True Strain | Ratio Strain wo/w salt | Stress Kpa | Ratio Stress wo/w salt | Fold Test Score |
|---|---|---|---|---|---|---|---|---|---|
| **Silver hake** (Merluccius bilinearis) | | | | | | | | | |
| w salt | 78.0 ± 0.1 | 6.9 | 76.8 ± 0.3 | 7.0 | 1.51 ± 0.13 | 0.74 | 38.1 ± 6.2 | 0.75 | 5 |
| wo salt | 78.0 ± 0.1 | 6.9 | 79.4 ± 0.2 | 6.8 | 1.12 ± 0.15 | | 28.7 ± 4.9 | | 3 |
| **White hake** (Urophycis tenuis) | | | | | | | | | |
| w salt | 82.3 ± 0.1 | 7.2 | 80.0 ± 1.3 | 6.9 | 1.64 ± 0.14 | 0.79 | 49.6 ± 5.2 | 0.87 | 5 |
| wo salt | 82.3 ± 0.1 | 7.2 | 81.6 ± 0.5 | 6.9 | 1.30 ± 0.07 | | 43.2 ± 3.3 | | 3 |
| Plearonectidae **Black back flounder** (Pseudopleuronectes americanus) | | | | | | | | | |
| w salt | 79.7 ± 0.3 | 6.7 | 81.3 ± 0.4 | 6.5 | 1.64 ± 0.06 | 0.80 | 22.7 ± 3.0 | 0.76 | 5 |
| wo salt | 79.7 ± 0.3 | 6.7 | 80.1 ± 0.4 | 6.5 | 1.32 ± 0.09 | | 17.3 ± 1.1 | | 3 |
| **Dab** (Hippoglossoides platessoides) | | | | | | | | | |
| w salt | 83.6 ± 0.3 | 7.0 | 83.6 ± 0.5 | 6.8 | 1.50 ± 0.18 | 0.85 | 17.9 ± 1.8 | 1.21 | 5 |
| wo salt | 83.6 ± 0.5 | 7.0 | 84.7 ± 0.0 | 6.8 | 1.27 ± 0.08 | | 21.7 ± 2.3 | | 5 |
| **Yellow tail flounder** (Limanda ferrunginea) | | | | | | | | | |
| w salt | 80.7 ± 0.5 | 6.8 | 82.7 ± 0.5 | 6.6 | 1.34 ± 0.08 | 0.82 | 18.3 ± 1.7 | 0.95 | 5 |
| wo salt | 80.7 ± 0.5 | 6.8 | 84.9 ± 0.9 | 6.6 | 1.10 ± 0.06 | | 17.3 ± 6.4 | | 3 |

Table 1 (continued)

| Species | Fish %$H_2O$ | Fish pH | Mince %$H_2O$ | Mince pH | True Strain | Ratio Strain wo/w salt | Stress Kpa | Ratio Stress wo/w salt | Fold Test Score |
|---|---|---|---|---|---|---|---|---|---|
| Pomatomidae | | | | | | | | | |
| **Bluefish** | | | | | | | | | |
| (Pomatomus saltatrix) | | | | | | | | | |
| [Whole muscle] | | | | | | | | | |
| w salt | 76.6 ± 0.1 | 6.0 | 77.4 ± 0.6 | --- | 1.49 ± 0.13 | 0.47 | 65.1 ± 6.4 | 0.57 | 5 |
| wo salt | 76.6 ± 0.1 | 6.0 | 79.3 ± 0.3 | --- | 0.70 ± 0.03 | | 36.9 ± 4.7 | | 3 |
| [White muscle] | | | | | | | | | |
| w salt | 74.9 ± 0.4 | 5.9 | 74.5 ± 0.0 | --- | 1.33 ± 0.08 | 0.47 | 93.4 ± 7.0 | 0.61 | 5 |
| wo salt | 74.9 ± 0.4 | 5.9 | 78.2 ± 0.7 | --- | 0.63 ± 0.10 | | 57.1 ± 4.5 | | 3 |
| Scombridae | | | | | | | | | |
| **Atlantic mackerel** | | | | | | | | | |
| (Scomber scombrus) | | | | | | | | | |
| [Whole muscle] | | | | | | | | | |
| w salt | 69.2 ± 0.1 | 6.1 | 73.3 ± 0.3 | 6.4 | 1.40 ± 0.08 | 0.48 | 75.8 ± 5.5 | 0.71 | 5 |
| wo salt | 69.2 ± 0.1 | 6.1 | 72.6 ± 0.1 | 7.1 | 0.67 ± 0.07 | | 53.6 ± 1.8 | | 2 |
| [White muscle] | | | | | | | | | |
| w salt | 76.2 ± 3.1 | 6.1 | 72.7 ± 1.1 | 6.4 | 1.59 ± 0.26 | 0.50 | 85.0 ± 13.7 | 0.63 | 5 |
| wo salt | 76.2 ± 3.1 | 6.1 | 74.8 ± 1.6 | 6.3 | 0.80 ± 0.08 | | 53.4 ± 8.2 | | 2 |

There was a significant difference ($p \leq 0.05$) between stress and strain values in the presence or absence of salt for all species except for stress values for Atlantic pollock, cod, cusk, and yellow tail flounder where the differences were not significant ($p > 0.05$).

the corresponding ratios. As in the previous section on the Survey of Species, the experiments always represent a direct comparison of washed minced muscle to one half of which 3% NaCl was added, while the other had no salt added beyond the 0.15% used for washing. Table 2 gives moisture contents, pH values and fold test scores of the washed minced muscle samples. Although there were some changes in the absolute values of true strain with different samples from different months of the year, the ratio of true strain of samples without added salt compared to that in the presence of added salt was relatively steady except for a dip in June. The red hake spawning season is June through early summer (Bigelow and Schroeder 1953); thus, this seasonal change could be a reflection of spawning.

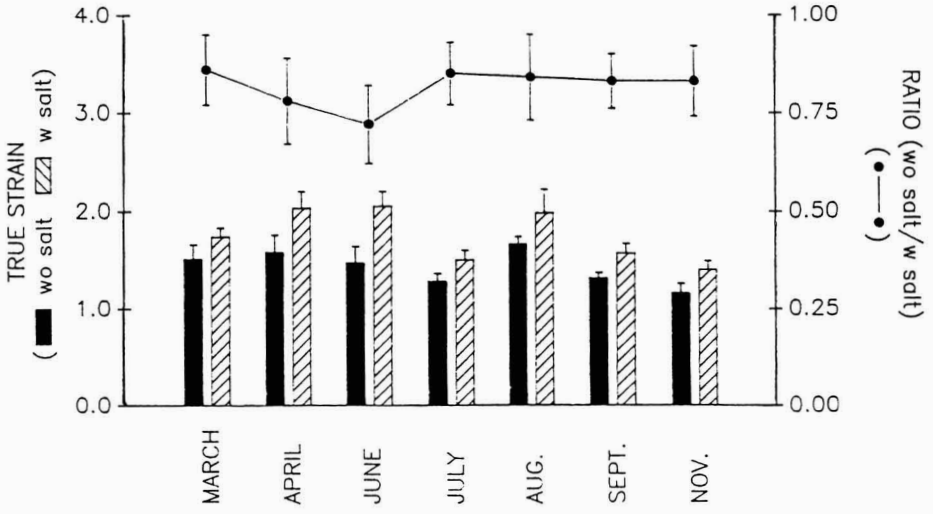


FIG. 1. SEASONAL VARIATIONS (1989) IN TRUE STRAIN VALUES OF RED HAKE GELS MADE WITH HIGH AND LOW SALT CONCENTRATIONS

Absolute values of stress varied more than did the true strain although the ratio was fairly constant and greater than 1.0 for 5 of the 7 months. There was a drop in the ratio below 1.0 in June and March with the greater drop occurring in June. The results did not seem to be related in a simple way to either moisture content or pH of the samples (Table 2).

In the presence of added 3% NaCl, red hake samples from all of the 7 months yielded a score of 5 in the fold test. Samples from September and November scored a 5 in the fold test without the addition of the extra salt. Another sample prepared in October and not shown here also had a score of 5

in the absence of high salt. However, at other times of the year these scores were lower, dropping to 3.5 in March.

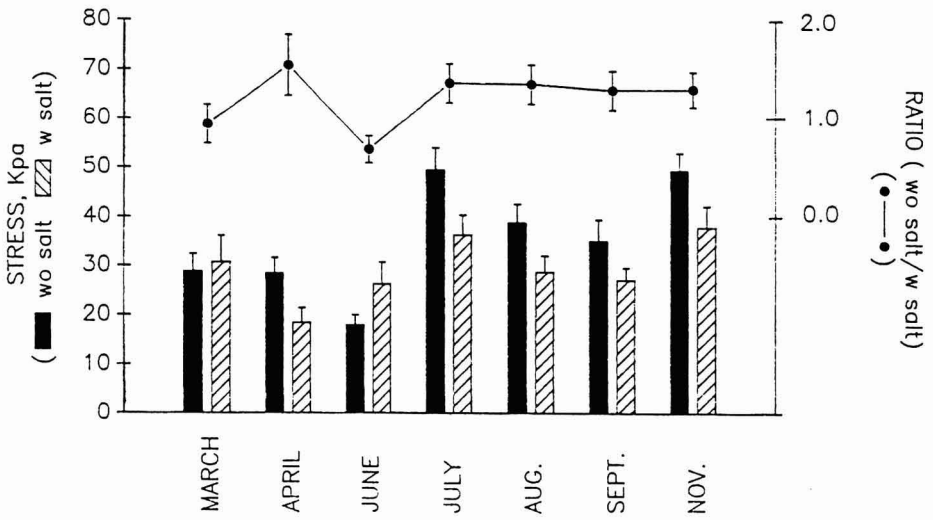


FIG. 2. SEASONAL VARIATIONS (1989) IN STRESS VALUES OF RED HAKE GELS MADE WITH HIGH AND LOW SALT CONCENTRATIONS

TABLE 2.
SOME SEASONAL PROPERTIES OF RED HAKE MINCE AND GELS

| 1989, Month | washed mince | | fold test score | |
|---|---|---|---|---|
| | % moisture | pH | high salt | low salt |
| March | 83.6 ± 0.3 | 6.7 | 5 | 3.5 |
| April | 86.6 ± 1.1 | - | 5 | 4.1 |
| June | 73.9 ± 0.4 | 6.8 | 5 | 4.5 |
| July | 83.6 ± 2.9 | 6.6 | 5 | 4.2 |
| August | 85.1 ± 0.0 | 6.5 | 5 | 4.6 |
| September | 81.5 ± 0.1 | 6.6 | 5 | 5 |
| November | 79.7 ± 0.5 | 6.7 | 5 | 5 |

Shimizu and Wendakoon (1990) examined seasonal variations in the gel-forming ability and chemical composition of lizardfish muscle. They found that the gel-forming ability decreased during the spawning period of June and July; there was also a decrease in myofibrillar protein and an increase in

myosin-degrading activity at 50C and 60C. Gel-forming ability remained low immediately after spawning because of a continued high level of myosin-degrading activity.

## Role of Cryoprotectants in Gelation Properties

In the earlier experiments gels were formed in the absence of a cryoprotectant mixture to reduce interfering factors in the evaluation of the role of NaCl on gel formation. However, since most fish gels are in fact produced in the presence of cryoprotectants, it was thought useful to determine the role of cryoprotectants on the ability to form gels in the absence of high levels of NaCl. Red hake was the species chosen to investigate this property. All samples were stored for 1 week at −20C. With cryoprotectants the true strain ratios were higher without salt with ratios of 1.10, 1.15, and 1.18 in the three experiments conducted (Table 3). In the case of the sample with the true strain ratio of 1.15 in the absence compared to the presence of added salt, the ratio obtained with the same sample of fish in the absence of cryoprotectants was 0.95.

TABLE 3.
COMPARISON OF LOW- AND HIGH-SALT GELS FROM RED HAKE IN THE PRESENCE OF CRYOPROTECTANTS

| Date of experiment | True Strain | Stress, Kpa |
|---|---|---|
| 4/19/90 | | |
| with salt | 2.01 ± 0.12 a | 16.6 ± 2.2 a |
| without salt | 2.22 ± 0.13 b | 30.2 ± 3.4 b |
| 5/2/90 | | |
| with salt | 1.63 ± 0.17 a | 29.1 ± 3.0 a |
| without salt | 1.87 ± 0.07 b | 47.8 ± 3.6 b |
| 5/16/90 | | |
| with salt | 1.69 ± 0.12 a | 12.7 ± 1.6 a |
| without salt | 1.99 ± 0.10 b | 22.9 ± 1.7 b |

Means within a column (for each experiment) that have a different letter (ab) are significantly different ($p < 0.01$). All samples gave fold test scores of 5.0.

The stress ratios in the gels made without salt compared to those made in the presence of 3% NaCl were high, ranging between 1.64 and 1.82. The values of stress of gels made in the absence of cryoprotectants averaged 1.34, which was in the range previously found for fish caught in the same season (April and May) in the previous year (Fig. 2). Thus, the relative value of both

true strain and stress of gels prepared in the absence of added salt increased relative to those prepared in the presence of added salt when cryoprotectants were added. The differences were statistically significant ($p < 0.01$).

The reason for this effect of cryoprotectants is not known, but the surimi was stored at −20C for a relatively short period of time. Compounds like sugars and phosphates protect proteins from denaturation under a variety of stress conditions (Findlay and Barbut 1992; Konno 1992) and not just freezing and thawing. Some denaturation during heating is thought to be necessary for gel formation. It is possible that excessive denaturation may adversely affect gel formation. The cryoprotectants may have prevented the excessive denaturation, thus allowing stronger and more elastic gels.

In the experiments of Table 1, many factors were not controlled. These include preprocessing factors such as season and on-board handling and processing variables like moisture content and pH. Thus, it is important to recognize that only the differences caused by the presence or absence of the 3% added salt are entirely meaningful. Nevertheless, there did appear to be a species effect as we had earlier observed (Hennigar *et al.* 1988, 1989). Seasonal effects on strain and stress values of gels prepared from red hake were greater in absolute values than in the ratios obtained in the absence and presence of salt. The ratios were relatively constant except near the spawning season. Unexpectedly, true strain values were greater in the absence of added salt than with added salt when cryoprotectants were used and the samples stored frozen for one week. It is possible that this is a reflection of the destabilizing effect of high concentrations of NaCl (Kelleher and Hultin 1991), which was manifested both in the freezing process and during heating to form the gels.

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# INTERESTERIFICATION REACTIONS CATALYZED BY A LIPASE IMMOBILIZED ON A HYDROPHOBIC SUPPORT

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## ABSTRACT

*Bacterial lipase from* Pseudomonas cepacia *immobilized on a hydrophobic support was used to bring about the interesterification reactions of the triglycerides of olive oil with octanoic acid. When the lipase was immobilized on microporous polypropylene powder, the overall reaction rate was approximately seven times faster than that observed when the support was diatomaceous earth or when a comparable amount of free enzyme was present in the reaction mixture. At water contents of less than 1% by weight, an increase in the concentration of water resulted in faster rates of interesterification at the expense of a larger accumulation of intermediate hydrolysis products such as mono- and diglycerides. In the solventless interesterification reactions of octanoic acid and the triglycerides of olive oil, a higher ratio of octanoic acid to olive oil resulted in both faster initial rates of interesterification and eventually, as the mixture approached equilibrium, a higher fraction of octanoic acid residues in the glycerides of olive oil.*

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## INTRODUCTION

The increased availability of triacylglycerol lipases (EC3.1.1.3) has made it possible for researchers to employ the catalytic properties of lipases in innovative ways. The use of lipases in organic media is of particular interest to researchers trying to develop new products from natural fats and oils. In aqueous solution, lipases will usually hydrolyze one or more of the ester bonds of triglycerides, producing diglycerides, monoglycerides, or glycerol and the corresponding number of free fatty acids. However, when the same lipase is used in an organic solution containing triglycerides, free fatty acid, and a very small amount of water (usually less than 1% by weight), the free fatty acid is inserted into the triglyceride at the expense of a fatty acid residue originally present in the triglyceride molecule. This type of reaction is termed an interesterification reaction (Macrae 1985). One well-known application of this reaction is the production of a substitute for cocoa butter by the interesterification reactions of stearic acid and the triglycerides of the mid fraction of palm oil (Macrae 1985; Bloomer *et al.* 1990).

The research described in this article is intended to contribute to the database for assessment of the technical and economic implications of using an immobilized lipase to modify triglycerides. A long term goal of our laboratory is to develop technologies for modification of the triglycerides of milkfat and other natural fats and oils. Via interesterification reactions with the appropriate fatty acids, one can, in principle, generate value-added products whose potential markets range from butter substitutes and food flavorings for the general market to triglycerides tailored for use in the production of specialty foods targeted at the health conscious consumer (e.g., the therapeutic foods that are increasingly being referred to as nutriceuticals or pharmafoods).

Attempts to develop processes for the production of tailor-made foods should start from a clear understanding of the kinetics of the interesterification reaction and a knowledge of how the rates of the various reactions constituting the reaction network are affected by such process variables as the nature of the enzyme support, the fluid/solid and fluid/fluid interfacial areas, the enzyme loading, the reaction temperature, the concentrations of substrates and water, etc. Olive oil was selected as a model system for milkfat in order to simplify the chemical analyses necessary to elucidate the kinetics of the reactions involved. The fatty acid residues present in the triglycerides of olive oil are also abundant in milkfat. However, milkfat also contains six additional types of fatty acid residues that constitute a significant fraction of the overall fatty acid composition. Consequently, it is easier to perform the necessary chemical and mathematical analyses on the olive oil system. Furthermore, olive oil is a liquid at room temperature, a situation that facilitates both the handling of the reaction mixture and sample processing. Octanoic acid was chosen as the fatty acid to be

exchanged because it is believed that the human body metabolizes fatty acids having less than 12 carbon atoms in a manner similar to that by which carbohydrates are metabolized (Megremis 1991). Therefore the contribution of these short chain fatty acids to health problems associated with the cardiovascular system is thought to be minimal.

The affinity of lipases for hydrophobic interfaces constitutes an essential element of the mechanism by which these enzymes act. Researchers have proposed that surface binding activates or enhances the catalytic activity of lipase (Brockman *et al.* 1988). When lipase immobilized on a nonpolar support was used to hydrolyze olive oil, it displayed better activity and stability than when other traditional supports (e.g., diatomaceous earth) were used (Brady *et al.* 1986). Since the interesterification reaction of interest takes place in a hydrophobic medium, the additional hydrophobic character imparted to the microenvironment of the lipase when it is immobilized on a nonpolar support ought to influence the kinetics of this reaction. These considerations were important in selecting microporous polypropylene as the support for the immobilized lipase. Reports in the literature regarding the use of hydrophobic supports for lipases are rare, and they are usually concerned with the applications of these enzymes to hydrolysis reactions (Brady *et al.* 1988), not with the biocatalysis of interesterification reactions.

## MATERIALS AND METHODS

### Materials

A nonspecific lipase from the bacterial source *Pseudomonas cepacia* (Amano International, Troy, VA) was used without further purification. The supports employed were a microporous polypropylene powder, less than 250 $\mu$m in diameter (Accurel powder, Akzo Chemicals, McCook, IL, and Enka AG, W. Germany) and diatomaceous earth (Celite 545, Fisher Scientific, Chicago, IL). The substrates for the interesterification reaction were olive oil and octanoic acid (both from Aldrich, Milwaukee, WI). The internal standards used in the HPLC analysis were heptadecanoic acid and nonanoic acid (both obtained from Sigma Chemical Co., St. Louis, MO). Butylated hydroxyanisole (BHA) was used as an antioxidant in the fatty acid solutions (Sigma). Materials used for sample preparation were: 2,4′-dibromoacetophenone (PBPB), 18-crown-6 ether, formic acid (all from Aldrich), and potassium carbonate (Mallinckrodt, Paris, KY). Analyses of the water content of the olive oil-octanoic acid mixtures and of the immobilized enzyme were carried out using a single-reagent Karl Fischer titrant (Aldrich). Reagents used in the analysis for total fatty acids include: sodium

hydroxide and sulfuric acid, $BF_3$-butanol (14% w/w) (Alltech Associates, Deerfield, IL), and anhydrous sodium sulfate (Mallinckrodt). The solvents used in lipase precipitation, washing, sample preparation, and HPLC separations were the following: acetone, chloroform, methanol, hexane (all obtained from VWR, Chicago, IL), acetonitrile (VWR and Sigma), butanol, pentane (both from Aldrich), and ethyl ether (Sigma).

All chemicals were reagent grade or better and were used without further purification. The water employed was processed by reverse osmosis and then deionized before use.

## Procedure for Immobilization of the Lipase

A 1.5 g portion of lipase was dissolved in 100 ml of 1 mM citrate buffer, pH 7, and centrifuged for 5 min at 2,500 rpm to separate any insoluble material. The supernatant was poured into a beaker and 4.5 g of the polypropylene (PP) powder were added slowly while mixing with a magnetic stirrer. The PP powder was allowed to disperse in the lipase solution for 5 to 10 min and the beaker was then placed in an ice bath. It was allowed to equilibrate with the bath for 15 min, during which time stirring was maintained. A 150 ml portion of chilled acetone was then added very slowly to precipitate the lipase onto the PP powder. The mixture was then filtered with a Büchner funnel and allowed to air-dry in the funnel for 2 h. The dried PP-lipase powder was subsequently vacuum dried for an additional 2 h at room temperature in a Speed Vac Concentrator, model SVC-100H (Savant Instruments, Farmingdale, NY). The amount of immobilized protein is equal to the difference between the weight of the vacuum dried material and the weight of the PP support.

## Procedure for the Interesterification Reaction

The interesterification reaction was carried out in a 120 ml glass reactor provided with a rubber stopper and a water jacket designed to maintain the temperature of the vessel constant with the aid of a Haake D8 temperature controller (Haake, Saddle Brook, NJ). The interesterification reactions were carried out at temperatures of 40 and 50C in the absence of any solvent. Appropriate amounts of olive oil and octanoic acid were mixed in a beaker with rapid stirring.

One means of providing water to the reaction mixture involved addition of about 3 ml of 1 mM, pH 7 citrate buffer to the olive oil-octanoic acid (OO/OA) mixture to achieve saturation of the oil phase. (The presence of a small quantity of water is necessary to effect the hydrolysis reaction, which constitutes the first step in the process of interesterification.) The mixture was then centrifuged to separate the water and oil phases. Usually 65 ml of the resultant solution would

then be poured into the reactor. If a higher level of water concentration was desired, from 0 to 350 μl of buffer would be added to the PP-lipase powder. The powder was then poured into the reaction mixture to initiate the reaction. Duplicate samples were collected at selected time intervals over a period of 9–28 h (depending on the initial reaction conditions).

## Sample Preparation and HPLC Analysis

The samples were centrifuged at 2,500 rpm for 2 min to separate the solid phase from the oil phase. The procedure employed for sample preparation and HPLC analysis is a modification of a procedure developed by Reed *et al.* (1984). A 50 μl portion of the oil phase was collected and mixed with 4.5 ml of a 1:1 chloroform-methanol solution containing 1 g/L of the internal standards (heptadecanoic and nonanoic acids) and 0.05% BHA. A 250 ml portion of the resultant solution was mixed with a solution containing 1 ml of 1 g/L of PBPB in acetonitrile, 50 μl of 5 g/L crown ether in acetonitrile, 2.5 ml of additional acetonitrile, and 0.2 g of potassium carbonate. This mixture was heated to 75–80C and held at this temperature for 30 min. Then 40 μl of 40 g/L formic acid in acetonitrile were added. The solution was held at the same temperature for another 5 min. This solution was refrigerated at 4C for at least 1 h, then cold-filtered through a 0.45 μm membrane to separate any precipitated glycerides.

The HPLC analysis was performed at ambient temperature using a Waters 600E chromatograph equipped with a 700 Satellite WISP autosampler and a 490E UV detector. The data were collected and analyzed using Waters' Maxima 825 chromatography software in a NEC Power Mate 1 computer. The chromatographic separation required injection of a 20 μl sample to a Waters 15 cm long Resolve column with 5 μm spherical packing. The flow rate was 1.8 ml/min; the solvents were acetonitrile and water. The convex gradient consisted of the following steps: (a) 55–90% acetonitrile for 10 min; (b) 90–100% acetonitrile for 3 min; (c) 100% acetonitrile for 5 min. The separated components were detected at 254 nm.

## Characterization of the Feedstock

The average molecular weight of the feedstock olive oil was determined by performing an analysis for total fatty acids via gas chromatography. This step was necessary because the HPLC procedure employed does not separate palmitic and oleic acids to the desired degree. A 150 mg sample of olive oil was added to 4 ml of 0.5 N sodium hydroxide in butanol. The solution was boiled at 85C for 10 min. Then, 1 ml of an internal standard solution containing 650 ppm nonanoic acid in ethyl ether was added together with 0.5 ml of 5.5 N sulfuric

acid and 1 ml of $BF_3$-butanol. This solution was boiled for another 10 min and then extracted with 15 ml of a water-methanol solution (39:6, v/v) and 15 ml of pentane. This mixture was centrifuged and the upper organic layer was extracted with another 15 ml of the water-methanol solution. The organic layer was separated and 1 g of anhydrous sodium sulfate was added thereto. A 3–5 $\mu$l aliquot of this solution was injected into a Hewlett Packard 5890A gas chromatograph equipped with a split injector, an FID detector and 15 ft of a Supelco (Carbowax) capillary column. The output of the detector was collected using an Apple IIe computer and analyzed with the aid of Chromatochart software (Interactive Microware, State College, PA). The operating conditions were as follows: the carrier gas was helium, flowing at 2 ml/min; air and hydrogen were fed to the FID at 472 and 31 ml/min respectively; the split ratio was 13.5; the initial and final temperatures were 60C and 220C, respectively; the heating rate was 10C/min; both the injector and the detector were held at 220C.

### Analysis for Water Content

The amounts of water present in either the OO/OA mixture or in the vacuum dried PP-lipase powder were determined using a Karl-Fischer titration. The samples (5 ml of oil mixture or 1 g of powder) were analyzed in a Fisher model 391 K-F Titrimiter (Pittsburgh, PA). Methanol and chloroform were used as the solvents. A single-reagent titration was performed.

## RESULTS AND DISCUSSION

### Immobilization

The immobilization procedure is an adaptation of a procedure developed by Wisdom *et al.* (1987) for the immobilization of lipase on diatomaceous earth (Celite) by acetone precipitation. The present support, microporous polypropylene, was chosen because of its hydrophobic character. Brady *et al.* (1986) have previously used microporous polypropylene as a support, but their immobilization procedure involved conventional adsorption. The data presented in Table 1 permit one to compare the three immobilization procedures. Using Wisdom's acetone precipitation technique, one can immobilize more lipase onto the polypropylene than is obtained if the enzyme is merely allowed to "spontaneously" adsorb on the surface of the polymer. The difference in loading can be as much as a factor of four.

TABLE 1.
METHODS FOR IMMOBILIZATION OF LIPASE

| Immobilization method | Support | % Lipase loading (wt protein/wt Support) | Lipase source | Reference |
|---|---|---|---|---|
| Adsorption | Microporous polypropylene | 8.1 | *Candida* | Brady et al. 1986 |
| Acetone precipitation | Celite | 3.1 - 11.1 | *Aspergillus* | Wisdom et al. 1987 |
| Acetone precipitation | Microporous polypropylene | 13.1 - 34.0 | *Pseudomonas* | Present work |

## Comparison of Supports for the Interesterification Reactions

The interesterification activity of lipase from *Pseudomonas cepacia* was studied in three different microenvironments: (1) free lipase; (2) lipase immobilized on Celite; (3) lipase immobilized on microporous polypropylene. The data in Fig. 1 indicate the dependence of the extent of incorporation of octanoic acid into the glycerides of olive oil for each of the three cases indicated above. In all cases the initial reaction conditions were the same and the concentrations of enzyme were comparable. While the free lipase and Celite-lipase systems were characterized by very similar reaction rates, the interesterification activity of the PP-lipase system was about seven times greater during the initial stages of the reaction. There are two possible (nonmutually exclusive) explanations of the observed behavior. First, the interaction between the lipase and the hydrophobic surface of the PP powder might be activating the enzyme. The results of Brockman *et al.* (1988) indicate that lipases have an adsorption site that is different from the catalytic site. X-ray analyses of lipases by Brady and coworkers (Brady *et al.* 1990) have revealed the existence of a surface loop that covers the active site of the enzyme. According to these investigators, this surface loop could be displaced from the active site when a lipase is activated by interfacial interactions, i.e., the surface loop could move in a manner that uncovers the active site. The use of polypropylene as a support may facilitate displacement of this surface loop. Second, since both olive oil and octanoic acid are nonpolar liquids, adsorption of lipase on a hydrophobic support could provide these reactants with easier access to the active site of the enzyme.

When dealing with immobilized enzymes, one always needs to consider the possibility that diffusion or mass transfer processes may influence the observed kinetics of the reaction. However, in the present study these phenomena are

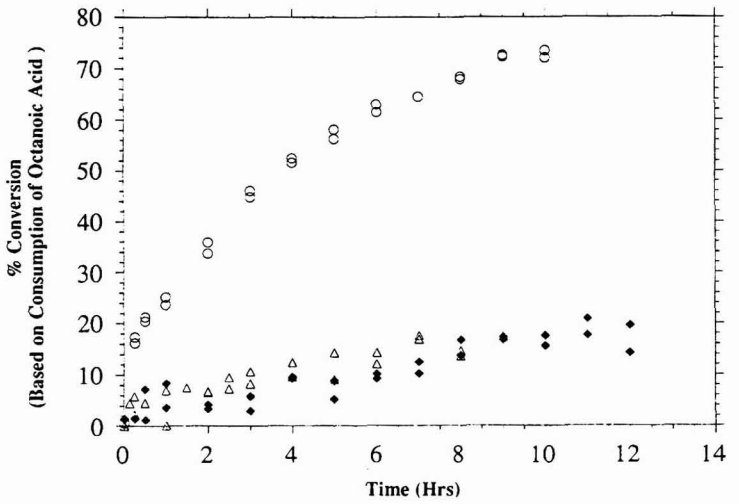


FIG. 1. INTERESTERIFICATION OF OLIVE OIL WITH OCTANOIC ACID AS CATALYZED BY THE LIPASE FROM *PSEUDOMONAS CEPACIA* IN THREE DIFFERENT MICROENVIRONMENTS

The molar ratio of octanoic acid to olive oil was 1:2. Support materials: ○, 1.2 g of lipase immobilized on microporous polypropylene; ◆, 1.32 g of lipase immobilized on Celite; Δ, 1.2 g of free lipase.

unimportant in view of the fact that both the system containing the free lipase and that with the enzyme immobilized on Celite exhibited similar reaction rates. The microporous polypropylene powder used as the hydrophobic support had a diameter of less than 250 $\mu$m. The rates of the observed reactions are much slower than the calculated rates of the diffusion and mass transfer processes associated with transport of reactants and products into and out of supports with the indicated dimensions. Thus the PP-lipase system was not expected to suffer from mass transfer limitations on the reaction rate.

## Thermal Stability

When used in an aqueous solution to hydrolyze fats, lipases have a thermal stability limit in the vicinity of 40C (Kang and Rhee 1989; Kwon *et al.* 1987). Above 40C, lipases rapidly lose activity (half-lives are of the order of hours). However, under reaction conditions in which the amount of water present is extremely low, lipases can display sustained interesterification activity even at temperatures as high as 100C (Zaks and Klibanov 1984). The data depicted in Fig. 2 indicate that the PP-lipase system retained interesterification activity during 2 consecutive runs at 50C. There was no apparent loss of activity after a combined reaction time of 28 h. In fact, in the second run the PP-lipase

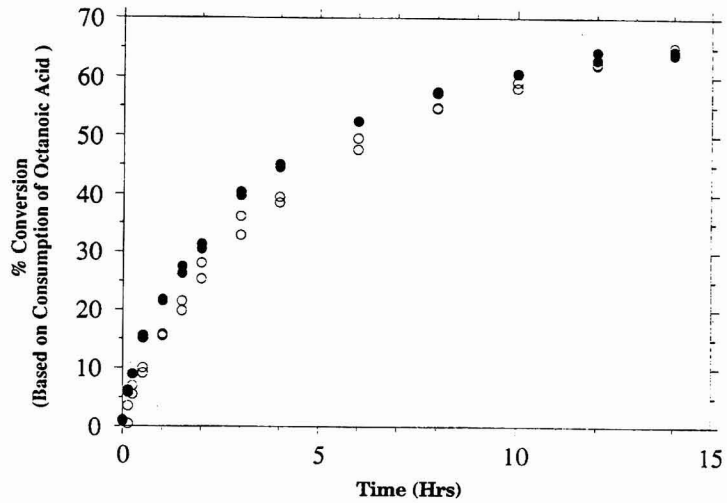


FIG. 2. RETENTION OF THE ACTIVITY OF LIPASE FROM *PSEUDOMONAS CEPACIA* IMMOBILIZED ON POLYPROPYLENE POWDER WHEN SUBJECTED TO REPEATED USE The temperature was 50C. The effective concentration of water was 178 mM. Octanoic acid and olive oil were present in a molar ratio of 1:1. ○, first reaction; ●, second reaction.

seemed to exhibit a slight increase in activity. This result may be attributable to the fact that the PP-lipase was thoroughly washed with hexane to remove the glycerides and fatty acids present after the first run. The PP-lipase powder dispersed very readily in the hexane, since the latter is a hydrophobic liquid. Visual inspection of the PP-lipase powder subsequent to the washing procedure indicated that the initial extent of agglomeration of the powder was significantly less than that prevailing for the same powder prior to its use in the first run. Nevertheless, in both cases the agglomerates disappeared once the powder was dispersed in the reaction mixture for a sufficient time (after 15–30 min of reaction). Reduction of the extent of agglomeration facilitates access of reactants to the immobilized enzyme and can lead to slightly enhanced rates of reaction. These enhancements may result from a situation in which disintegration of the agglomerates provides access to regions of the solid, which were previously rendered inaccessible by virtue of physical blockage of pore mouths. Since agglomeration was observed only during the first few minutes of the reaction, its effect on the reaction is believed to be minimal given that the total reaction time was usually more than 10 h. It should also be noted that at long times in both runs, the reaction mixtures tended to approach the same asymptotic level of conversion.

## Effects of Water Content and Octanoic Acid Concentration

In order for the interesterification reaction to take place, a minimum amount of water must be present to permit production (through hydrolysis) of the intermediate glycerides (e.g., diglycerides or monoglycerides) (Macrae 1985) that are subsequently able to react with octanoic acid. The data in Fig. 3 indicate the influence of two different initial effective concentrations of water on the conversion profile. (The term effective concentration of water refers to the total amount of water available in both the oil phase and the PP-lipase system per unit volume of the oil phase.) When the effective water concentration was increased from 238 mM to 448 mM, there was an increase in the initial rate of the interesterification reaction. Since the interesterification reaction is limited by thermodynamic equilibrium constraints, a greater amount of water present at the beginning of the reaction will produce greater initial rates of interesterification at the expense of a small reduction in the total amount of octanoic acid that can eventually be incorporated into the olive oil. Inspection of Fig. 3 indicates that after 20 h of reaction the level of octanoic acid in the mixture having the lower concentration of water begins to approach that for the mixture having the higher concentration.

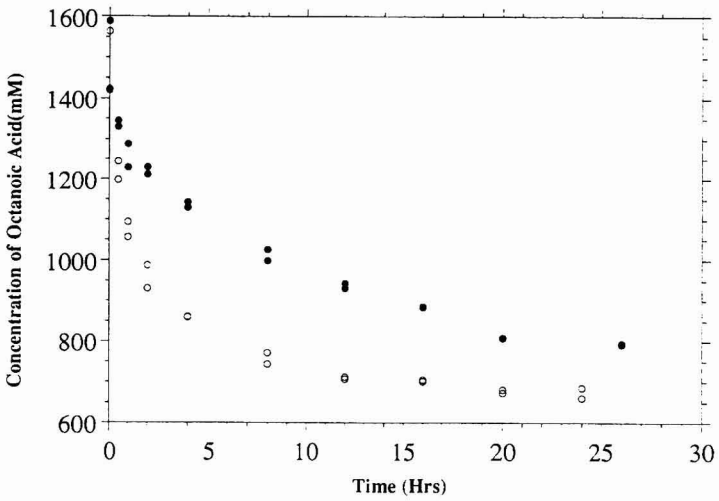


FIG. 3. EFFECT OF INITIAL WATER CONTENT ON INTERESTERIFICATION ACTIVITY AT 40C
Effective water concentration: ●, 238 mM; ○, 449 mM.

If more water is present at the beginning of the reaction, the initial rate of hydrolysis must increase (i.e., the rate of formation of intermediate glycerides

increases)(Goderis *et al.* 1987). Other analytical data collected in our laboratory (not reported here) indicate that the mixture having the higher concentration of water is characterized by both faster initial rates of hydrolysis and a larger accumulation of liberated fatty acids.

The concentration of free hydroxyl moieties of the intermediate glycerides (and available for reesterification with free octanoic acid) can be calculated from stoichiometric considerations as:

$$IG = FA - [(C8)_0 - C8] \quad (1)$$

where IG = concentration of free hydroxyl moieties
FA = concentration of fatty acids released from olive oil
$(C8)_0$ = initial concentration of octanoic acid
C8 = concentration of octanoic acid

At the end of the reaction, the value of IG corresponds to the amount of by-products formed. If one employs an average molecular weight of 875 for the olive oil (obtained from GC analysis), the percentage of the original ester bonds that have not been reesterified after 24 h is 17.5% for the mixture with a water concentration of 238 mM and 26% for that in which the water level is 448 mM. A higher initial concentration of water will accelerate the rate of interesterification, but it also will lead to higher yields of di- and monoglycerides (and perhaps some glycerol).

If the concentrations of the intermediate glyceride species are important in determining the rate at which octanoic acid is substituted for other fatty acid residues in the glycerides of olive oil, then the relative concentration of the coreactant octanoic acid should also have a comparable effect. The data in Fig. 4a indicate the influence of two different levels of octanoic acid on the interesterification rate. Since no solvent was used, a change in the concentration of octanoic acid implies a concomitant change in the concentration of olive oil. Consequently, the different reaction conditions are compared on the basis of different ratios of octanoic acid to olive oil (mole/mole). It is apparent that the mixture having the higher ratio (1.88) consumes significantly more octanoic acid during the first hour of reaction than the mixture having the lower ratio (0.45).

Cursory inspections of yields of the reaction based on the fractional conversion of octanoic acid can be misleading. For the experiments described in this paper, octanoic acid is the limiting reagent because there are three reactive sites per triglyceride molecule. When the molal ratio of octanoic acid to olive oil was 0.45, about 75% of the initial octanoic acid was consumed within 24 h. When this ratio was 1.88, almost 55% was consumed within 24 h (see Fig. 4b). On the other hand, a somewhat different picture emerges if one looks at the fraction of the ester bonds that originally were available for

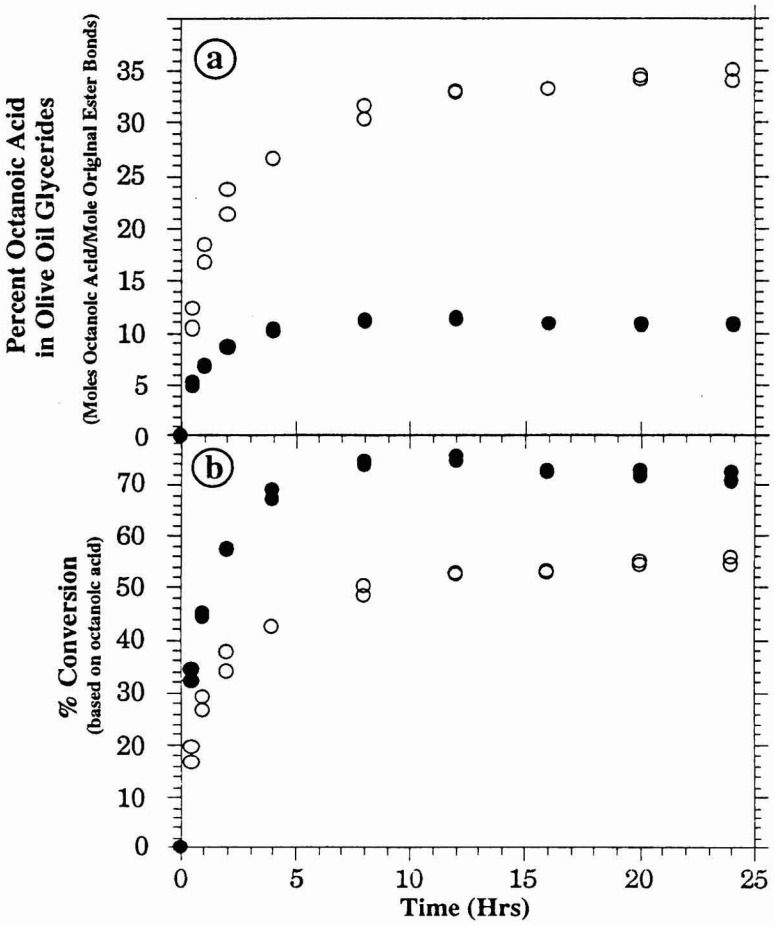


FIG. 4. EFFECT OF THE RELATIVE CONCENTRATION OF OCTANOIC ACID ON INTERESTERIFICATION ACTIVITY AT 40C

(a) Incorporation of octanoic acid into the glycerides of olive oil; (b) conversion of octanoic acid. The molar ratios of octanoic acid to olive oil employed were: ○, 1.88; ●, 0.45.

interesterification. (The concentration of these bonds is equal to three times the molar concentration of olive oil.) When the molal ratio of octanoic acid to olive oil was 1.88, almost 35% of the original total number of ester bonds participated in the interesterification reactions. When this ratio was 0.45, the corresponding number was only 11% (see Fig. 4a). Thus a larger mole ratio of octanoic acid to olive oil both accelerates the interesterification reaction and enriches the octanoic acid composition of the glycerides of olive oil to a greater extent.

## ACKNOWLEDGMENT

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# MATHEMATICAL MODELING OF TRANSIENT HEAT AND MASS TRANSPORT IN A BAKING BISCUIT

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## ABSTRACT

*Drying behavior of a single baking biscuit was modeled using unsteady state, anisotropic, two dimensional, simultaneous heat and mass balances. Solutions of these equations agreed well with the experimentally determined temperature and the moisture data. Modeling revealed that in the outer sections of the baking biscuit conduction and diffusion were the dominant heat and mass transfer mechanisms, respectively. In the central section of the biscuit the gas cells cracked with the increased vapor pressure and the upward volume expansion, then air/vapor enclaves were formed among the horizontal dough layers in the radial direction. The dominant heat and mass transfer mechanisms in the central section of the biscuit were convection. Presence of two different regime zones in a baking biscuit may have important consequences concerning the strength of the commercial products against crumbling during marketing and consumption.*

## INTRODUCTION

Heat effects create a number of physical and chemical changes in a biscuit during baking including gas formation, denaturation and coagulation of proteins, gelatinization of starch, crust formation, browning reactions, evaporation of water, expansion of volume, and development of porous structure (Smith 1966). Although the dough may be homogeneous at the beginning of a baking process,

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it undergoes nonuniform physical and chemical changes, thus modeling of temperature and water distribution requires simultaneous solution of two dimensional, unsteady state, asymmetric, anisotropic heat and mass transfer equations. Anisotropy and asymmetry result from nonuniform heating and subsequent structural changes. Influence of structural changes on internal thermal conductivity and moisture diffusivity is important. Apparent thermal conductivity of solids have to be established experimentally since they depend on many factors that are difficult to measure or predict theoretically, such as the pore size, fluid, i.e., liquid water or vapor, contained in the pores, etc. (Bird *et al.* 1960). These same limitations are also valid for diffusion in solids. Thermal conductivity and diffusivity during drying are usually estimated as regression parameters of the models describing the temperature or moisture profiles in the drying object and the existing equations for thermal conductivity and diffusivity are limited mostly to empirical correlations (Bakshi and Yoon 1984; Christenson *et al.* 1989; Tong and Lund 1990; Karathanos *et al.* 1990). Simultaneous heat and mass transfer equations have been extensively used for modeling the drying phenomena (Harmathy 1969; Young 1969; Husain *et al.* 1973). Baking is a very sophisticated process when compared to drying, and such equations have not been extensively applied to modeling of baking yet.

In a baking process the surface is first preheated from the initial temperature to the boiling temperature. In the second phase evaporation occurs at the surface at constant boiling temperature. This second phase is similar to the constant rate period in drying literature. In the third phase of the process water transport rates from the depths of the solid become less than the rate of evaporation at the surface, thus surface temperatures exceed the boiling temperature (Skjöldebrand and Hallström 1980). The third phase is called the falling rate period in the drying terminology. In a baking process the surface may attain the boiling temperature immediately, and water is transported to the surface mainly with diffusion as long as the constant rate drying prevails. Since dehydration of the baked goods is not generally desired, the falling rate period does not always occur.

There are gas cells in the dough formed by the air bubbles either occluded in the dough during mixing or adsorbed on the flour particles. Gluten, the protein complex of the dough, is stretched into a viscoelastic film and makes the walls of the gas cells. In fermented dough products, carbon dioxide produced by the yeast diffuses into these cells, causing volume change (Ponte and Tsen 1978; Akdoğan and Özilgen 1992; Yöndem *et al.* 1992). A similar mechanism of volume expansion with fermented bakery products may also be present in the nonfermented products, where water vapor, instead of carbon dioxide, may accumulate in the cells, increase the total pressure, and cause volume expansion. An increase in water content in the central region during the baking process was reported by Skjöldebrand and Hallström (1980). Since only the top of the pan

is open, the dough expands in the longitudinal direction; therefore only the sides of the gas cells are stretched upwards and channels (cracks) form in these stretched regions, allowing flow of the water vapor in the radial direction. In the inner region of the baking biscuit, convection may occur through these cracks in addition to diffusion. Convection is much faster than conduction; therefore the regions where heat and mass transfer occurs with convection may attain uniform radial temperature and moisture profiles rapidly.

Mathematical models help to understand the details of the processes, which may not be understood by plotting the data only. In the present study a mathematical model will be developed for heat and mass transfer in a baking biscuit.

## MATHEMATICAL MODEL

Radial and axial migration of water in the cylindrical outer layers of biscuit (either in the upper or lower half of the biscuit) is described as:

$$\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{D}{1-X}\frac{\partial X}{\partial r}\right)+\frac{\partial}{\partial Z}\left(\frac{D}{1-X}\frac{\partial X}{\partial Z}\right)=\frac{\partial X}{\partial t} \tag{1}$$

The first and the second terms on the left represent diffusion of water in the radial and axial directions, respectively. When the moisture contents of the biscuits are high the term $(1 - X)$ may not be neglected. The term $\delta X/\delta t$ gives variation of the water content, at a fixed point, as a function of time. Figure 1 shows that in the radial direction $r = 0$ is next to the pan, the diffusion dominated cylindrical region is $0 \leq r \leq R_t$ and the convection region is between is $R_t \leq r \leq R$. In the longitudinal direction $Z = 0$ describes either the top or the bottom of the biscuit and h/2 describes the plane dividing the biscuit into two equal parts. The boundary conditions of Eq. 1 are:

$$X = X_{EQ} \text{ when } t \geq 0 \text{ and } Z = 0 \text{ (for all r, at the top)} \tag{2a}$$

$$X = X_S(0) \text{ when } t \geq 0 \text{ and } Z = 0 \text{ (for all r, at the bottom)} \tag{2b}$$

$$X = X_S(Z) \text{ when } t \geq 0 \text{ and } r = 0 \text{ (for all Z, next to the pan)} \tag{3}$$

$$\frac{dX}{dZ} = 0 \text{ when } Z = h/2 \quad \text{(in the middle of the pan)} \tag{4}$$

Initial condition for Eq. 1 is:

$$X = X_0 \text{ when } t = 0, \text{ for all Z and r.} \tag{5}$$

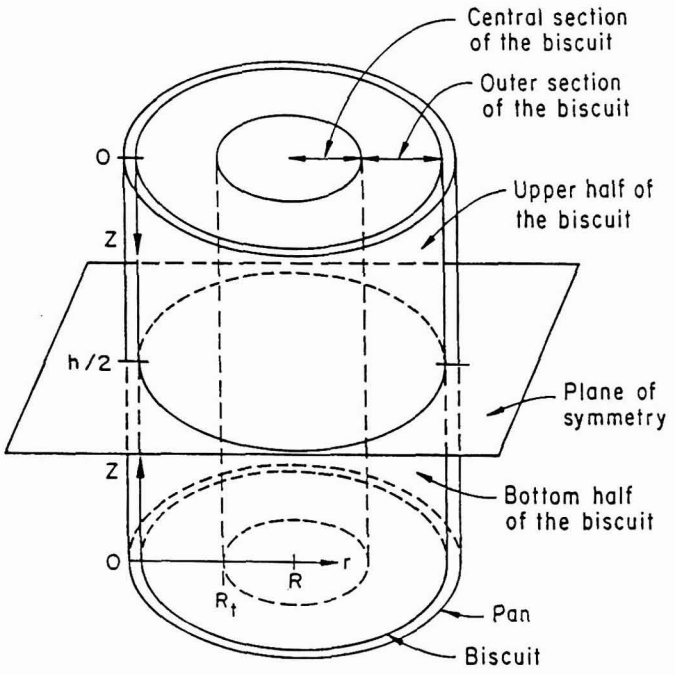


FIG. 1. SCHEMATIC DIAGRAM OF THE BAKING BISCUIT
(Not in actual size.)

Equation 2a implies that moisture equilibrium is established between the top of the biscuit and the oven as soon as baking starts, and prevails until the end of the process. There was not perfect air-tight interface between the pan and the biscuit. It is suggested that there were interstitial air spaces, connected with channels, between the pan and the biscuit. Water vapor diffusing in the biscuit in the radial direction (or in the longitudinal direction at the bottom) enters into these spaces (after leaving the biscuit) then diffuses in the air until leaving the pan. Equations 2b and 3 show that due to the presence of high amounts of vapor in these spaces local equilibrium is established at different surface moisture contents. The surface moisture content $X_S(0)$ was a constant, but $X_S(Z)$ varied with location. Both of $X_S(0)$ and $X_S(Z)$ did not change with time at fixed locations. Equation 4 implies that there was no water transport between the lower and the upper parts of the pan. Equation 5 indicates that the moisture content of the biscuit was initially uniform and constant.

Temperature variation in the cylindrical outer layers of the biscuit (either in the upper or lower half of the biscuit) is described as:

$$k\frac{\partial^2 T}{\partial r^2} - \frac{k}{r}\frac{\partial T}{\partial r} + k\frac{\partial^2 T}{\partial Z^2} = \rho_s (C\,T + \lambda)\frac{\partial X}{\partial t} + \rho C\frac{\partial T}{\partial t} \tag{6}$$

Terms on the left give the radial and axial heat transfer in the cylindrical volume element. The term $\rho_s(C\ T + \lambda)\ \delta X/\delta t$ accounts for loss of sensible and latent heat via loss of water, and $\rho C\ \delta T/\delta t$ refers to temperature change. Boundary conditions for Eq. 6:

$$T = T_B \text{ when } t \geq 0 \text{ and } Z = 0$$
(for all r, at the top or the bottom of the pan) (7)

$$T = T_B \text{ when } t \geq 0 \text{ and } r = 0$$
(for all Z, next to the pan) (8)

$$\frac{dT}{dZ} = 0 \text{ when } Z = h/2 \quad \text{(in the middle of the pan)} \tag{9}$$

Initial condition for Eq. 6:

$$T = T_0 \text{ when } t = 0, \text{ for all Z and r.} \tag{10}$$

Equations 7 and 8 suggest that at the air/biscuit interfaces (at the top bottom or sides) boiling temperature prevails throughout the baking process. Equation 9 implies that there is no heat transfer between the lower and the upper halves of the pan. Equation 10 indicates that the temperature of the biscuit was initially uniform and constant.

In the central section of the biscuit, where convection occurs in the cracks in radial directions water and temperature profiles may be described as:

$$\frac{dX}{dr} = 0 \text{ when } R_t \leq r \leq R \quad \text{(in the central section)} \tag{11}$$

$$\frac{dT}{dr} = 0 \text{ when } R_t \leq r \leq R \quad \text{(in the central section)} \tag{12}$$

In the present study Eq. 1–12 will be used for modeling the baking phenomena to understand the mechanisms of the heat and mass transfer in the baking biscuit.

## MATERIALS AND METHODS

The dough contained 50% white wheat flour, 25% olive oil and 25% water (w/w). The biscuits were baked at 200 ± 5C, in a cylindrical aluminum pan (5.0 cm ID, 2.5 cm deep), in a toaster oven. Initial weights of the biscuits were 60 g. Temperatures in the biscuits were monitored at four locations (r,Z) of (1.5,1.0); (2.5,1.0); (2.0,1.5); (1.0,1.3), using 0.125 mm diameter Teflon coated copper-constantan thermocouples, and a multichannel datalogger (Esterline Angus, PD2064, Esterline Corp., Indianapolis). Biscuits were baked one at a time for 5, 10, 15, 20, and 25 min. Surface properties and boiling on the surface were observed visually. Immediately after a baking period, one cm diameter cylindrical samples were taken from the center and side, next to the pan wall. Samples were cut to present the top, middle and bottom thirds of the dough cylinder (sample size for each region = 1.5 g). Water contents of the samples were determined gravimetrically, by vacuum oven drying for 12 h at 70C and $8 \times 10^3$ Pa pressure. Details of the experimental procedure and distribution of oil in the biscuit are also described elsewhere (Heil *et al.* 1993).

## RESULTS AND DISCUSSION

No crust formation was observed in this study. Evaporation (with boiling) was visually observed on the entire top surface until the end of baking (25 min). These observations confirmed that baking occurred mainly at the constant (drying) rate period. The central sections of the biscuits raised for about 20 min, reconfirming the increase of the pressure in this region. The rise of the central section may also confirm that the vapor could not leave the biscuit in the longitudinal direction. Almost uniform increase of the height of the biscuit in this region may imply that there was no pressure gradient in the radial direction, reconfirming the fast convection that equilibrated the radial pressure distribution immediately. The layered structure of the central section collapsed some time after 20 min of baking possibly due to loss of mechanical strength caused by gelatinization of starch. The biscuit did not stick to the pan, and surface irregularities were observed at the bottom and sides of the baked biscuit confirming the presence of the interstitial spaces between the biscuit and the pan.

Equations 1 and 6 are converted into difference equations and rearranged in Eq. 13 and 14, respectively:

$$X(i+1,j,n) = X(i,j,n) + \Delta t D\{\frac{1}{(1 - X(i,j,n))^2}\frac{X^2(i,j+1,n) - 2X(i,j+1,n)\,X(i,j-1,n) + X^2(i,j-1,n)}{4\Delta r^2} +$$

$$\frac{1}{1-X(i,j,n)}\frac{X(i,j+1,n) - 2X(i,j,n) + X(i,j-1,n)}{\Delta r^2} + \frac{1}{r(1 - X(i,j,n))}\frac{X(i,j+1,n) - X(i,j-1,n)}{2\Delta r} +$$

$$\frac{1}{1 - X(i,j,n)}\frac{X(i,j,n+1) - 2X(i,j,n) + X(i,j,n-1)}{\Delta Z^2} +$$

$$\frac{1}{(1 - X(i,j,n))^2}\frac{X^2(i,j,n+1) - 2X(i,j,n-1)\,X(i,j,n-1) + X^2(i,j,n-1)}{4\Delta Z^2}\} \qquad (13)$$

and,

$$T(i+1,j,n) = T(i,j,n) + \frac{\Delta t}{\rho_s[0.5 + X(i,j,n) + Y]C}\{k_R\frac{T(i,j+1,n) - 2T(i,,j,n) + T(i,j-1,n)}{\Delta r^2} -$$

$$\frac{k_R}{r}\frac{T(i,j+1,n) - T(i,j-1,n)}{2\Delta r} + k_Z\frac{T(i,j,n) - 2T(i,j,n) + T(i,j,n-1)}{\Delta Z^2} -$$

$$\rho_s(C\,T(i,j,n) + \lambda)\frac{X(i+1,j,n) - X(i,j,n)}{\Delta t}\} \qquad (14)$$

A similar procedure as that of Eq. 13 was used in a previous study for modeling drying of an apple slab (McCarthy *et al.* 1991), but the phenomena described in Eq. 13 is substantially more complicated than that of drying. Indexes were defined in Eq. 13 and 14 such that i = 1 when t = 0 min; j = 1 when biscuit volume element is next to wall of the pan; and n = 1, when volume element is either on top or bottom surface. The origins of the coordinate system described in Fig. 1 are not typically employed for the solution of problems with cylindrical geometry. The major reason for the unusual choice of the origins is the two regime nature of the problem. The boundary between the diffusion and conduction dominated regimes is not highly visible, and might be suggested only after comparing the solutions of Eq. 1 and 6 with the experimental data. The uncertainty about the boundary requires the numerical solutions to be started from the outer boundaries. Thermal conductivity (k) was expressed according to Young (1969):

$$k = K_1 + K_2\,X(i,j,n) \qquad (15)$$

Parameters $K_1$ and $K_2$ are the heat transfer coefficients of the nonaqueous and aqueous components of the biscuits, respectively. Equation 15 is actually a simplified empirical representation, where change of parameters $K_1$ and $K_2$ with temperature, conversion of liquid water into vapor and structural changes within the product are not considered.

An empirical expression for water diffusivity in the biscuits was given by Tong and Lund (1990):

$$D = K_3 \exp \{K_4 X(i,j,n) - \frac{K_5}{T(i,j,n)}\} \quad (16)$$

where $K_3$, $K_4$ and $K_5$ were adjustable constants. Values of parameters for Eq. 16 were: $K_3 = 0.019$ m$^2$/s; $K_4 = 0.45$ kg solids/kg water; and $K_5 = 5400$ K$^{-1}$. Equation 16 is actually an empirical equation and does not take the multiphasic nature, i.e., solid portions and gas cells, etc., into consideration. There are some structure based equations for diffusivity available in the literature (i.e., Vagenas and Karathanos 1991; Özilgen 1993), but they are not as easily applicable as Eq. 16, due to need for structural information in their use.

Equations 13 and 14 are initial value problems. They were used to calculate numerical values of parameters X(i+1,j,n) and T(i+1,j,n), after substituting: $\rho_s$ = 600 kg/m$^3$ (measured experimentally); C = 2.30 + 4.19 X(i,j,n) kJ/kg (2.30 = empirical constant, 4.19 = heat capacity of water kJ/kg); $\lambda$ = 2263 kJ/kg (latent heat of evaporation of water at the boiling point); Y = 0.5 kg oil/kg solids; $\Delta t$ = 0.5 min; and $\Delta r$ = 0.25 cm. Initial value of height increment was $\Delta Z$ = 0.208 cm, however, a factor of 0.001 (determined experimentally) was added with the elapse of each time increment for volume increase during the baking process. The boiling temperature of water was 103C (highest temperature measured experimentally after boiling was attained). Equations 13 and 14 were solved separately for the upper and lower halves of the biscuits. Temperature of each volume element remains constant after reaching the boiling temperature ($T_B$). Expressions for thermal conductivities were found by trial and error procedure to minimize the sum of squares error between the experimental data and the solutions of Eq. 14 as:

$$k_Z = 5.06 + 39.8 X(i,j,n) \quad (17)$$

$$k_R = 10.2 + 79.5 X(i,j,n) \quad (18)$$

Comparison of Eq. 17 and 18 shows that thermal conductivity was higher in the radial direction than in the axial direction. This might be caused by the structural differences of the biscuit along the radial and the longitudinal

directions. Equilibrium moisture contents ($X_{EQ}$) were estimated with the same technique as the heat transfer coefficients as 0.10 kg water/kg solids for the top, and $X_S(0)$ was 0.35 kg water/kg solids for the bottom of biscuit. At the wall of the pan, $X_S(Z)$ was assumed changing in linear manner between 0.10 and 0.35. Higher value of $X_S(0)$ for the bottom of pan compensated for accumulation of vapor at the biscuit-pan interface.

The model temperature profiles were plotted in Fig. 2 for three different distances from the top surface of the upper half of the biscuit. Predicted temperature profiles for the lower half of the biscuit were within 1% deviation range when compared with their symmetrical counterparts in the upper half. The conduction-dominated heat transfer regime prevailed in the radial distance range of $0 \leq r \leq 1.5$ cm, and the convection dominated range was at $1.5 \leq r \leq 2.5$ cm. The side surface of the biscuit attained the constant boiling temperature immediately. The temperature profiles of the conduction dominated range indicated sharp change, the convection dominated range had characteristic flat profiles. Figure 2 also shows that the temperature increase was faster in the regions close to the top surface. Conduction heat transfer and diffusion mass transfer occurred in the same volume elements of the biscuit. Water profiles had the reverse trend of the temperature profiles in this region (Fig. 2 and 3). Water profiles were flat in the convection dominated region (Fig. 3).

The moisture profiles were evaluated for the entire baking process after solving Eq. 13. The model predictions at the indicated locations were compared with the experimental data in Fig. 4. It should be noted here that the simulations in Fig. 4a–4f were from a single mathematical solution with a single set of model constants; therefore very good agreement may be claimed between the model and the data. The size of the samples used for determining the moisture and oil contents in various locations of the biscuit was small (approximately 1.5 g). Evaporation during sampling may be the actual source of the scatter of the data around the model (Fig. 4).

The temperature profiles were evaluated for the entire baking process after solving Eq. 14. The model predictions at the thermocouple locations were compared with the experimental data in Fig. 5. In addition to the temperature terms, Eq. 14 also has some moisture terms, i.e., $X(i+1,j,n)$ and $X(i,j,n)$, numerical equivalents of these terms were needed during the numerical solutions of Eq. 14 and were obtained from the previously obtained numerical moisture profiles (solutions of Eq. 13). It should be also noted here that the simulations in Fig. 5a–5d actually originated from a single mathematical solution of Eq. 14 with a single set of model constants, therefore almost perfect agreement may be claimed between the model and the data.

Modeling is used in the present study to understand the behavior of the baking biscuit under well-defined experimental conditions. Due to the extremely complex nature of the baking process any changes in the experimental

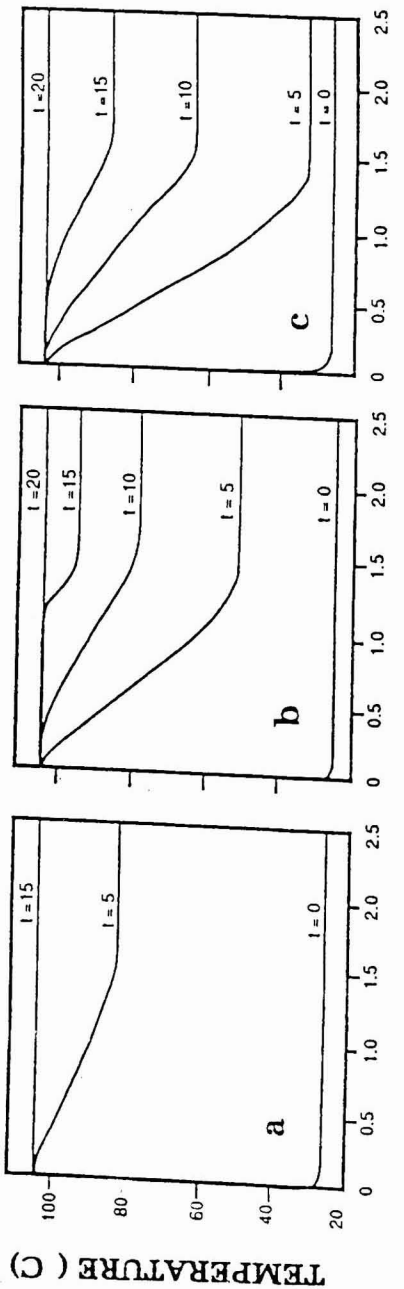


FIG.2. PREDICTED TEMPERATURE PROFILES
(a) 0.208 cm, (b) 0.624 cm, (c) 1.04 cm from the top (or bottom) surfaces.

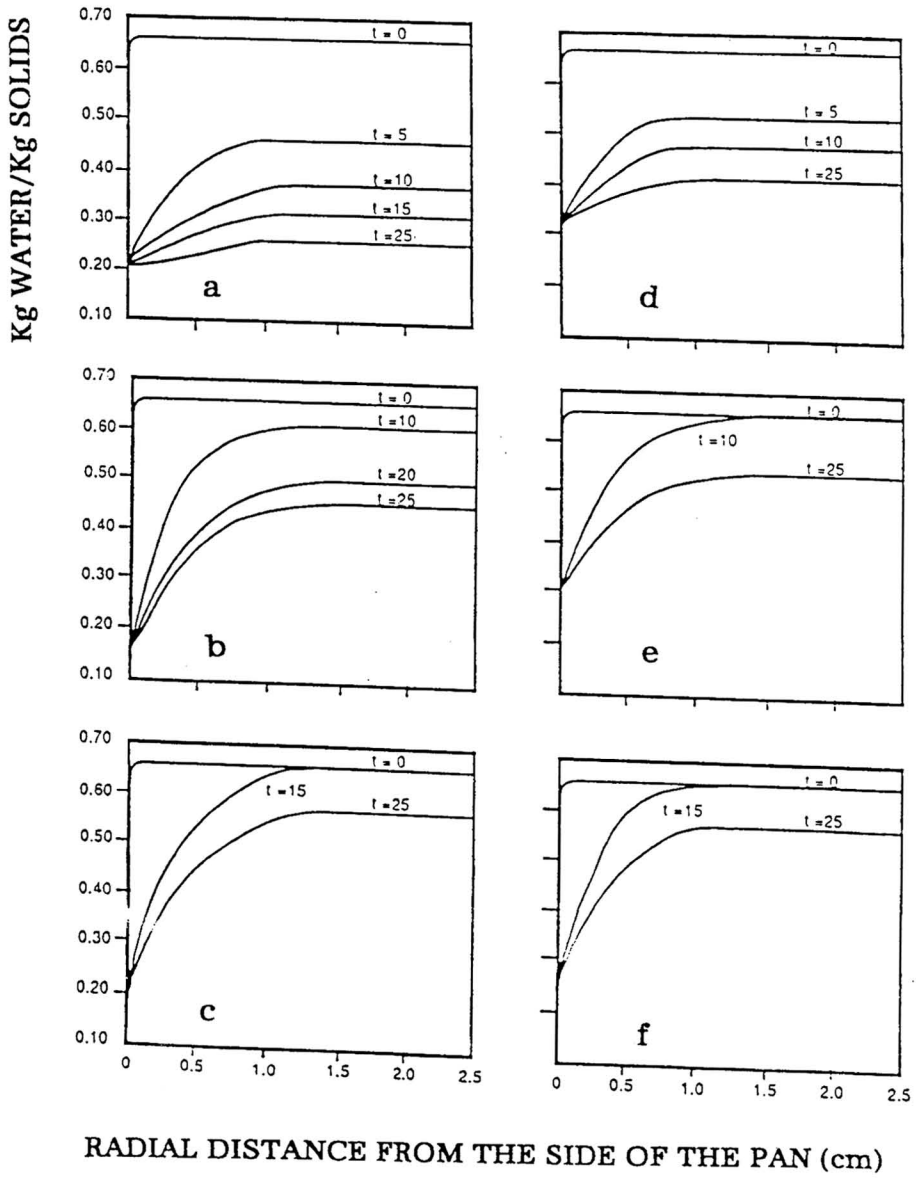


FIG. 3. PREDICTED MOISTURE PROFILES
(a) 0.208 cm, (b) 0.624 cm, (c) 1.04 cm from the top surface;
(d) 0.208 cm, (e) 0.624 cm, (f) 1.04 cm from the bottom surface.

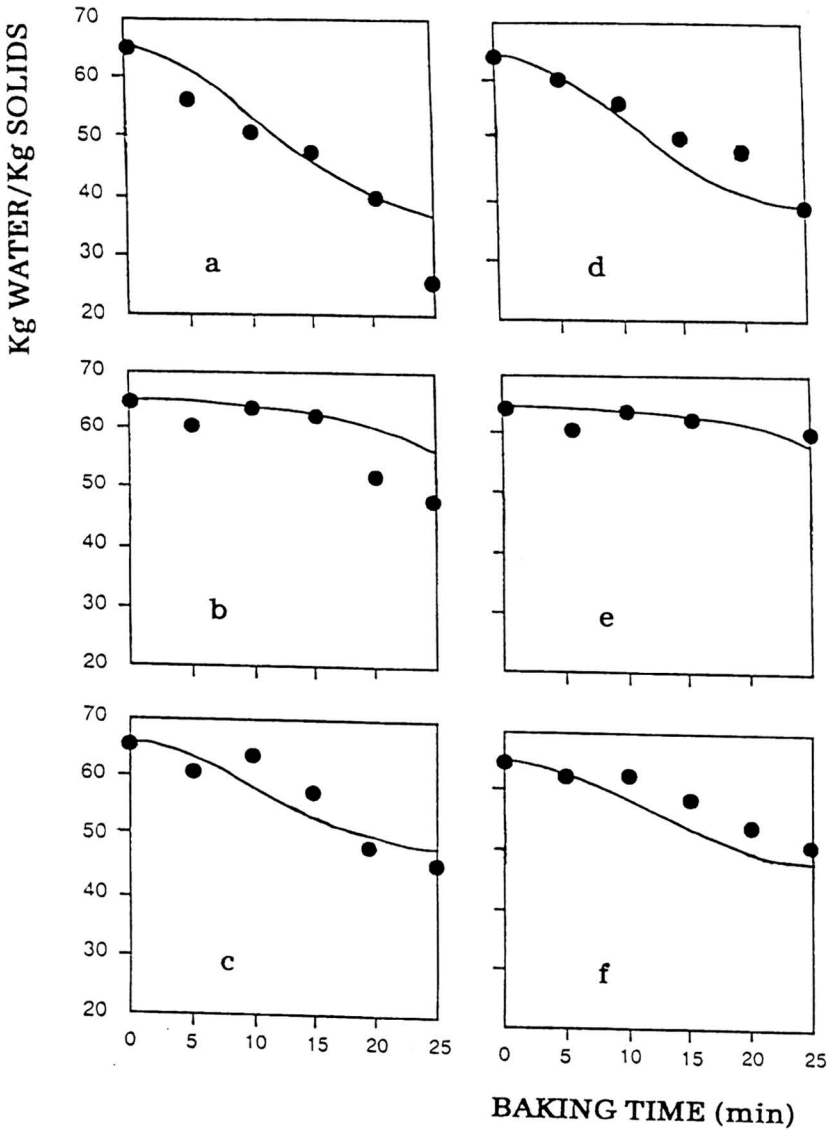


FIG. 4. COMPARISON OF THE MODEL MOISTURE CONTENTS (—) WITH THE EXPERIMENTAL DATA (●)
Upper half: (a) r = 1.25 cm, Z = 0.42 cm, (b) r = 1.25 cm, Z = 1.25 cm, (d) r = 2.5 cm, Z = 0.42 cm, (e) r = 2.5 cm, Z = 1.25 cm; bottom half: (c) r = 1.25 cm, Z = 0.42 cm (f) r = 2.5 cm, Z = 0.42 cm.

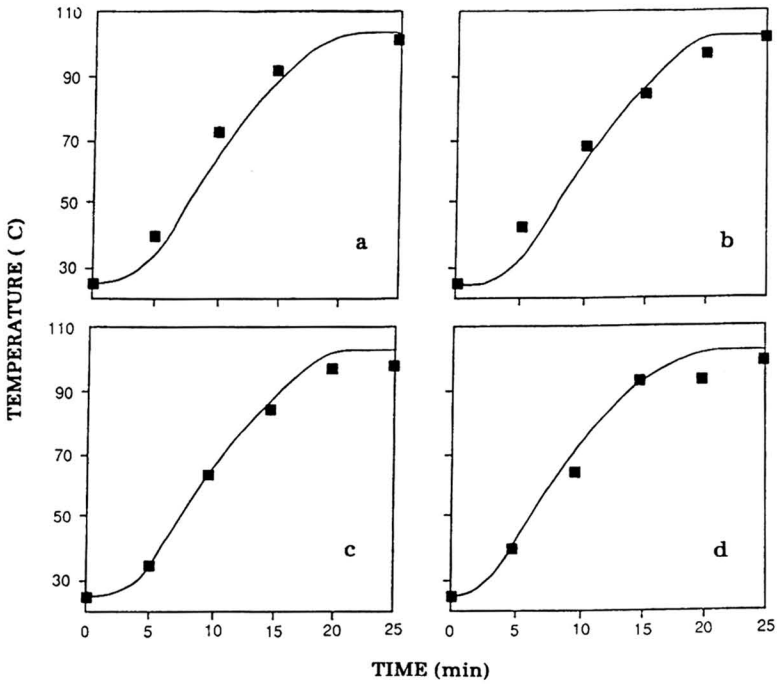


FIG. 5. COMPARISON OF THE MODEL TEMPERATURE PROFILES (—) WITH THE EXPERIMENTAL DATA (●)
(a) r = 1.5 cm, Z = 1.0 cm, (b) r = 2.5 cm, Z = 1.0 cm,
(c) r = 2.0 cm, Z = 1.5 cm, (d) r = 1.O cm, Z = 1.3 cm.

conditions, i.e., oven temperature (Turhan and Özilgen 1991), biscuit formulation, etc., may cause substantial changes in this behavior. Development of the convection zone in the biscuits is not desired in the industry. It occurs as a consequence of the damage in the gluten network, which substantially contributes to the strength of the biscuit against crumbling. It should be possible to confine convection domination to a very small range, or to totally eliminate it, by choosing an appropriate time-temperature profile. Using a high temperature period followed by a low temperature period is among the typical applications in the industry to decrease the internal vapor pressure. Damaging effect of the higher internal vapor pressure is reduced in the industry by drilling frequent holes through the thin biscuits. These holes aid in removing excess water from the central regions. They are made in fancy design also to make the product more attractive to the consumer.

## CONCLUSIONS

The modeling studies showed that there were two different regimes prevailing in the baking biscuit. The first regime prevailed in the outer sections of the cylindrical biscuit, where heat transfer was with conduction and moisture transfer was with diffusion. In the central section of the biscuit the dominant heat and mass transfer mechanisms were convection. There are gas cells in the dough surrounded with stretched viscoelastic gluten film. During the baking process, water vapor pressure increases in the central section of the dough and subsequently the gas cells are combined with each other, and a layered structure (horizontal dough layers separated with air/vapor enclaves) forms.

Our model has been based on extensive use of adjustable empirical model parameters. Values of these parameters may change in different experimental conditions. More research is needed to relate them to measurable properties and structure.

## ACKNOWLEDGMENTS

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## NOMENCLATURE

| | |
|---|---|
| C | Specific heat (kJ/kg K) |
| D | Apparent diffusivity of water in the biscuits ($m^2/s$) |
| h | Height of a biscuit (cm) |
| i | Time index (e.g., i = 1, time = 0) |
| j | Radial volume index (e.g., j = 1, volume element at side surface) |
| k | Heat transfer coefficient ($W/m^2$ K) |
| $k_R$ | Heat transfer coefficient in the radial direction ($W/m^2$ K) |
| $k_Z$ | Heat transfer coefficient in the axial direction ($W/m^2$ K) |
| $K_{1-5}$ | Constants |
| n | Axial volume index (e.g., n = 1, volume element on either top or bottom surface) |
| T | Temperature (C); also T(i,j,n) |
| $T_B$ | Boiling temperature (C) |
| $T_0$ | Initial temperature (C) |
| X | Moisture content of biscuit (kg water/kg dry solids); also X(i,j,n) |

$X_{EQ}$ Numerical values of parameter X(i,j,n), in equilibrium with the moisture content of air (kg water/kg dry solids)
$X_0$ Initial moisture content of biscuit (kg water/kg dry solids)
$X_S$ Numerical values of parameter X(i,j,n) at the side and bottom surfaces (kg water/kg dry solids)
R Radius of biscuit (cm)
$R_t$ Boundary of the transition from conduction to convection heat transfer or diffusion to convection dominated mass transfer regimes (cm)
r Radial distance from the side of a pan (cm)
$\Delta r$ Increment in the radial direction (cm)
t Time (min)
$\Delta t$ Time increment (min)
Y Oil content of the biscuit (kg oil/kg dry solids)
Z Axial distance from top (in the upper half) or the bottom surface (in the lower half) of the biscuit (cm)
$\Delta Z$ Increment in the axial direction (cm)
$\rho$ Density of the dough or the biscuit ($kg/m^3$)
$\rho s$ Density of the solids ($kg/m^3$)
$\lambda$ Latent heat of vaporization (kJ/kg)

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# SENSORY QUALITY OF IRRADIATED ONION AND GARLIC BULBS

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## ABSTRACT

*The radioinhibition process has shown to prolong shelf-life of "Valenciana sintética 14" onion variety and "Colorado" garlic variety. Sensory attributes of the irradiated bulbs were tested monthly by trained judges during extended storage in warehouse conditions (6–32C, R.H.40–50%). The sensory properties observed were external and internal appearance, firmness and odor.*

*The irradiated bulbs were judged to be superior in quality with respect to internal and external appearance ($p < 0.01$) and firmness ($p < 0.01$), after 180 days postharvest. The irradiated bulbs showed no difference in odor ($p < 0.05$), when compared to unirradiated ones, through the storage period.*

## INTRODUCTION

Argentina is an important producer of the highly marketable "Valenciana sintética 14" onion and the "Colorado" garlic. These varieties are produced in the region close to the Universidad Nacional del Sur. Considerable losses of garlic and onion bulbs occur during storage, mainly due to sprouting. The use of gamma radiation for the inhibition of sprouting in bulbs has been the object of several studies (Matsuyama and Umeda 1983; Thomas 1984).

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Extensive studies have been carried out in the Universidad Nacional del Sur, during eight consecutive harvests, to study the application of the radioinhibition process in the varieties mentioned above. A dose of 50 Gy of gamma rays is effective to extend the marketable period of onion and garlic bulbs, reducing weight loss and avoiding loss due to sprouting and rooting (Croci and Curzio 1983; Curzio and Croci 1983, 1988).

Argentina has had its own legislation since 1978, which was updated in 1988. The Ministry of Public Health authorized a series of market trials with these irradiated bulbs to evaluate consumer acceptance. The treatment applied to the products was described on tags attached to each bag as specified in the rules issued by the Argentina Food Code. The results of these tests showed that the consumers did not object to buying the irradiated bulbs. The consumers stated that the sensory quality of irradiated bulbs was better than the unirradiated ones (Curzio *et al.* 1986; Urioste *et al.* 1990).

Raw onion and garlic are widely used for culinary purpose as spices to improve the gastronomic properties of many foods. Irradiated onion and garlic must be of good appearance, firmness and flavor while on display in the retail outlets and in the consumer's home. Only very small sensory changes are permissible from the consumer's point of view. Several comparative studies of the sensory properties of unirradiated and irradiated bulbs have been carried out at the end of the storage period. The irradiated samples were judged to be superior in quality with respect to color, texture, odor and taste (Matin *et al.* 1985; Nair *et al.* 1973).

Since the radioinhibition process has been shown to prolong the shelf-life of onion and garlic bulbs, we decided to study the effects of relative low doses of ionizing radiation on sensory characteristics during long-term storage. This study is concerned with the evaluation of sensory properties of the irradiated "Valenciana sintética 14" onion variety and "Colorado" garlic variety during the storage period under simulated warehouse conditions. Thus, external and internal appearance, firmness and odor were tested monthly by trained judges.

## MATERIALS AND METHODS

### Specification of Products

Onion and garlic bulbs of the varieties "Valenciana sintética 14" and "Colorado", respectively, grown in the Southwest section of the province of Buenos Aires, were used in these studies. The bulbs were harvested in March (onion) and December (garlic) and cured naturally in the field within 10–20 days. About 200 kg of each of these bulbs were packed in nylon string bags (25 kg per bag).

## Irradiation and Storage

Half of the samples of each product were irradiated within 30–40 days postharvest at the facilities of the Comisión Nacional de Energía Atómica with an average dose of 50 Gy of $^{60}Co$ gamma rays. The dose uniformity ratio was 1.4. After irradiation, all the bulbs were stored under warehouse conditions (6–32C, R.H. 40–50%).

During storage (240 days postharvest for onion and 300 days for garlic), random samples of each treatment were taken monthly for sensory evaluations. Marketable bulbs (which did not show external sprouting, rotting and softening) were selected from random samples.

## Judges and Presentation of Samples

Trained judges were used as analytical instruments for testing samples of onion and garlic bulbs. The panel was composed of four females and two males, all university staff members. Training sessions were conducted a few days prior to the actual testing period to familiarize judges with test materials and sensory ballots.

Each product was evaluated six times during the storage period. The panelists worked individually in an area separate from the laboratory. Each month, six judges performed two sessions each (one day for the onion bulbs and another day for garlic bulbs and cloves).

## Evaluation of External and Internal Appearance

The order of presentation of the samples to each panelist was randomized and the bulbs were presented on plastic plates. Each judge received three pairs of samples coded with three digit random numbers. Each pair consisted of one irradiated sample and one unirradiated sample. At the end of the evaluation of each parameter the judgments were given in an individual questionnaire.

Simple paired comparisons tests were used (Larmond 1977) to evaluate the external appearance of the garlic cloves and onion bulbs. These tests were used to determine if there was a difference in external appearance between irradiated and unirradiated samples and to establish the direction of the difference.

The same procedure was used to evaluate the internal appearance of garlic cloves and onion bulbs. The garlic cloves and onion bulbs were presented in halves to the panelists, and were cut immediately before testing. The odor, color and general internal aspect were evaluated.

Significance levels for the proportions of judgments of simple paired comparisons were determined by binomial statistics (Roessler *et al.* 1978).

## Evaluation of Firmness

Unstructured scales with verbal anchors at the ends were used in the evaluation of firmness of the onion bulbs and garlic cloves (Larmond 1977). The scale consisted of a 10 cm horizontal line labeled "very soft" at the left end and "very firm" at the right end. Panelists were asked to make a vertical line across the horizontal line to indicate the magnitude of the firmness of the sample. Numerical values were given to the ratings by measuring the distance of the judges' marks from the left end of the line in units of 1.0 cm. A score of 5.0 or above was considered acceptable. The data were analyzed using ANOVA.

## Evaluation of Odor

Because of the overpowering odor of undiluted onion and garlic juice, the best method of assessing the relative odor intensity of different samples was to determine the threshold concentration in juice dilutions of these bulbs. The threshold concentration is defined as the minimum concentration of onion and garlic juice in water that can be detected by 70% of the judges (Schwimmer and Guadagni 1962).

Every month, three onion bulbs and three garlic bulbs from each treatment were peeled, weighed and then cut and triturated in a mixer with distilled water until a homogeneous solution of known concentration was obtained. The solutions were strained to clarify the solutions and eight dilutions of each product (four irradiated and four unirradiated) were prepared with odorless distilled water. The concentrations of such dilutions were 1, 2, 3 and 4 g/L of onion from each treatment and 0.02, 0.04, 0.06 and 0.1 g/L of garlic. Before preparing the dilutions, the approximate threshold range of onion in water was found to be 2–3 g/L and that of garlic 0.04–0.06 g/L. The different dilutions were put in sealed test tubes and were used to determine the olfactory threshold concentration as an index of the odor power of these bulbs. This parameter was determined with the duo-trio test (Larmond 1977).

Every month each panelist was given 16 triads, 8 of them corresponding to the different dilutions of onion juice and the other 8 corresponding to garlic juice. The panelists were given the samples with an increasingly higher concentration. Each triad consisted of a reference sample (odorless water) and two codified samples (one identical to the reference sample and the other containing the dilution of the juice). The panelists determined which of the codified samples was the same as the reference one.

# RESULTS

## External and Internal Appearance

The results of simple paired comparisons test for irradiated and unirradiated onion bulbs during storage are given in Table 1. From the beginning of storage (90 days postharvest) the judges found differences between the irradiated samples and the unirradiated ones in the pairs of analyzed bulbs ($p < 0.01$). As the judges pointed out that it can be seen from the table, in most pairs (10/15 and 9/15) the irradiated onion showed a better external and internal appearance. The preference for irradiated bulbs increased with longer storage periods, and for 180 days or more the judges preferred the bulbs of irradiated onion in all the analyzed pairs.

TABLE 1.
PROPORTION OF JUDGMENTS OF SIMPLE PAIRED COMPARISONS TEST WITH SIGNIFICANCE LEVELS FOR EACH TIME DURING STORAGE, INDICATING THE DEGREE OF PREFERENCE BETWEEN IRRADIATED AND UNIRRADIATED ONION BULBS, IN TERMS OF EXTERNAL AND INTERNAL APPEARANCE

| Storage time (days post-harvest) | External appearance | Internal appearance |
|---|---|---|
| 90 | 10/15 ( 0.154) | 9/15 ( 0.305) |
| 120 | 9/12 ( 0.075) | 10/15 ( 0.154) |
| 150 | 9/12 ( 0.075) | 10/15 ( 0 154) |
| 180 | 9/12 ( 0.075) | 15/15 (<0.001) |
| 210 | 15/15 (<0.001) | 15/15 (<0.001) |
| 240 | 15/15 (<0.001) | 15/15 (<0.001) |

Table 2 shows the results obtained in the evaluation of the external and internal appearance of garlic cloves. The results show that the judges found sensory differences between the cloves of garlic from both treatments throughout the storage period. The differences clearly showed the superiority of the irradiated cloves of garlic ($p < 0.01$).

TABLE 2.
PROPORTION OF JUDGMENTS OF SIMPLE PAIRED COMPARISONS TESTS WITH SIGNIFICANCE LEVELS FOR EACH TIME DURING STORAGE, INDICATING THE DEGREE OF PREFERENCE BETWEEN IRRADIATED AND UNIRRADIATED GARLIC CLOVES, IN TERMS OF EXTERNAL AND INTERNAL APPEARANCE

| Storage time (days post-harvest) | External appearance | Internal Appearance |
|---|---|---|
| 150 | 14/14 ( <0.001) | 13/15 ( 0.004) |
| 180 | 12/14 ( 0.006) | 12/15 ( 0.018) |
| 210 | 14/15 ( <0.004) | 12/15 ( 0.018) |
| 240 | 15/15 ( <0.001) | 12/15 ( 0.018) |
| 270 | 14/15 ( <0.004) | 15/15 (<0.001) |
| 300 | 13/15 ( 0.004) | 14/15 (<0.004) |

## Firmness

Table 3 summarizes the results of the firmness of control and irradiated onions. The irradiated onion bulbs keep their firmness through storage, whereas the unirradiated ones progressively lose their firmness. At the end of the storage period the unirradiated samples are unacceptable. The statistical analysis shows that from 180 days on there are significant differences in firmness ($p < 0.01$) in favor of the irradiated bulbs.

TABLE 3.
EFFECT OF IRRADIATION AND STORAGE ON THE FIRMNESS OF ONION BULBS

| Treatment | Storage time (days post harvest) | | | | | |
|---|---|---|---|---|---|---|
| | 90 | 120 | 50 | 180 | 210 | 240 |
| Unirradiated | 7.0±1.1 | 7.1±0.8 | 7.6±1.1 | 5.2±0.8* | 5.8±1.1* | 3.2±0.7* |
| Irradiated | 7.8±1.9 | 7.8±1.1 | 7.8±1.1 | 7.3±1.3 | 6.7±1.2 | 6.6±0.6 |

* Values differ significantly ($p < 0.01$)

Unstructured scale: 0 = very soft
5 = acceptable
10 = very firm

Table 4 shows the results obtained in the evaluation of firmness in garlic cloves. The results show that the irradiated cloves were firmer than the control cloves. The irradiated cloves of garlic kept their firmness until the end of the experiment. There is a significant decrease in firmness in the unirradiated cloves ($p < 0.01$) after 270 days postharvest.

TABLE 4.
EFFECT OF IRRADIATION AND STORAGE ON THE FIRMNESS OF GARLIC CLOVES

| Treatment | Storage time (days postharvest) | | | | | |
|---|---|---|---|---|---|---|
| | 150 | 180 | 210 | 240 | 270 | 300 |
| Unirradiated | 6.6±1.6 | 6.9±1.4 | 6.6±1.0 | 6.5±1.0 | 6.7±1.6 | 3.9±2.7* |
| Irradiated | 8.1±1.0 | 8.3±1.3 | 8.3±0.5 | 9.1±0.6 | 8.7±0.6 | 8.8±0.7 |

*Values differ significantly ($p < 0.01$)

Unstructured scale: 0 = very soft
5 = acceptable
10 = very firm

## Odor

Table 5 shows the results obtained in the determination of olfactory threshold concentration in irradiated and unirradiated onion bulbs during the storage period. No differences were found between the olfactory threshold concentration of irradiated and unirradiated bulbs. From 180 days of storage onwards, there is a reduction of the threshold concentration in both treatments.

Table 6 shows the results obtained in the determination of the olfactory threshold concentration in garlic bulbs. The values determined for both treatments coincide, except for 300 days postharvest when the threshold concentration of unirradiated bulbs is significantly higher than that of irradiated bulbs (Chi-square statistic, $p < 0.05$).

TABLE 5.
OLFACTORY THRESHOLD CONCENTRATION OF DILUTED ONION JUICE

| Storage time (days post-harvest) | Olfactory threshold concentration (g/L) | |
|---|---|---|
| | Unirradiated | Irradiated |
| 90 | 2 | 2 |
| 120 | 2 | 2 |
| 150 | 2 | 2 |
| 180 | 2 | 1 |
| 210 | 1 | 1 |
| 240 | 1 | 1 |

TABLE 6.
OLFACTORY THRESHOLD CONCENTRATION OF DILUTED GARLIC JUICE

| Storage time (days post-harvest) | Olfactory threshold concentration (g/L) | |
|---|---|---|
| | Unirradiated | Irradiated |
| 150 | 0.06 | 0.06 |
| 180 | 0.06 | 0.06 |
| 210 | 0.06 | 0.06 |
| 240 | 0.06 | 0.06 |
| 270 | 0.06 | 0.06 |
| 300 | 0.04 | 0.02* |

Values differ significantly ($p < 0.05$)

## DISCUSSION

After 180 days postharvest the unirradiated bulbs presented had suffered significant sensory quality loss. The sensory quality of irradiated onion and garlic bulbs remained constant and the products were judged acceptable through the end of the testing period. The irradiated bulbs were judged to be of superior internal and external appearance and firmness and showed no difference in odor when compared to unirradiated bulbs.

These results confirm that the radioinhibition process keeps the sensory quality of onion and garlic bulbs. This finding is supported by the fact that the gamma ray treatment delays the senescence of these bulbs (Croci 1988).

Irradiation of onion and garlic bulbs has proven to be an efficient means of prolonging shelf-life and reducing postharvest loss (Curzio and Croci 1990). The results of this sensory study makes the use of irradiation technology even more appealing.

## ACKNOWLEDGMENTS

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# CHEMICAL AND BACTERIOLOGICAL STABILITY OF FRANKFURTERS EXTENDED WITH WHEAT GERM, CORN GERM AND SOY PROTEINS[1]

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## ABSTRACT

Frankfurters extended with wheat germ, soy and corn germ proteins were tested for their storage stability. Water activity, bacteriological quality, total volatile nitrogen (TVN), ammonia nitrogen and thiobarbituric acid (TBA) values of samples stored at 3–4C for 45 days were evaluated. Extenders influenced storage stability of samples. Treatments were not different in their water activity. Total psychrotrophs increased upon storage up to log 5 CFU/g. Overall mean values for TVN of samples containing soy flour were higher while all samples showed an increase in TVN values upon storage. Extenders did not influence ammonia nitrogen content of samples, though an increase was observed upon storage. TBA values of samples were affected by extenders, showing an initial increase and subsequent decrease. In spite of these differences, all samples can be considered acceptable up to 45 days of storage at 3–4C. Samples containing WGPF were satisfactory in their storage stability.

## INTRODUCTION

Processing of comminuted meat products (CMP) and coarse ground meat products (CGMP) lend these products to be extended with nonmeat additives. A variety of different nonmeat additives are incorporated into CMP and CGMP as functional ingredients, to improve water binding, emulsifying capacity, emulsion stability, nutritional quality and yield (Comer 1979; Wills and Kabirullah 1981; Mittal and Usborne 1985; Parks and Carpenter 1987; Lin and Zayas 1987). Several studies involving shelf-life of meat products containing plant proteins reported an influence of plant proteins on the microbial growth

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resulting in undesirable changes in the product (Stansbury 1975; Foster *et al.* 1978; Thompson *et al.* 1978; Ziprin *et al.* 1981). Safety of CMP is more critical than intact meat cuts, such as steaks or roasts, which for various reasons provide better conditions for microbial growth, such as increase in surface area due to chopping, comminution, and grinding, increased contact with equipment during processing, incorporation of nonmeat additives, and longer refrigerated storage.

Oxidation of lipid component, proteolysis due to bacterial growth, subsequent deamination and decarboxylation of amino acids produce undesirable changes in texture and deterioration of flavor. These changes have a profound effect on acceptability of these products besides rendering them unsafe for consumption (Draughon 1980). Storage stability of CMP is a critical factor when evaluating a new protein additive.

Storage stability of meat products is usually evaluated by monitoring microbial growth, oxidative lipid rancidity and proteolysis of proteins as expressed in changes in the nitrogenous compounds. Availability of water is absolute requirement for bacterial growth and hence in CMP, water activity during storage is reliable criterion to evaluate shelf-life.

Defatted wheat germ protein flour (WGPF) is a milling by-product with a potential to be a good nonmeat additive in meat products. WGPF has been shown to compare well with soy flour (SF) and corn germ protein flour (CGPF) as an additive in CMP (Gnanasambandam and Zayas 1992). Studies were conducted on storage stability of frankfurters extended with CGPF (Zayas and Lin 1989), and as compared with milk proteins (Hung and Zayas 1991). However, information on storage stability of frankfurters containing WGPF has not been reported.

The objective of this study was to evaluate storage stability of frankfurters extended with WGPF as compared to those containing SF and CGPF. Tests conducted on storage stability of frankfurters included: water activity, total psychrotrophic counts, total volatile nitrogen, ammonia nitrogen, and thiobarbituric acid (TBA) values.

## MATERIALS AND METHODS

WGPF (R-80) was obtained from Vitamins, Inc., Chicago, IL, and SF (soy fluff 200W) from Central Soya Company, Fort Wayne, IN. CGPF was obtained from dry corn milling industry. Flow chart of frankfurter preparation is shown in Fig. 1. Meat (beef trim and boneless picnic 50/50) was ground through a 9.38 mm plate, mixed, reground through 4.69 mm plate and stored at −20C in sealed vacuum packages. Fat content in the blend of beef and boneless picnic was adjusted to 20%, and the batch size was 100 lb. Meat was thawed at 4C for 18 h before processing. Formulations containing additives were processed with

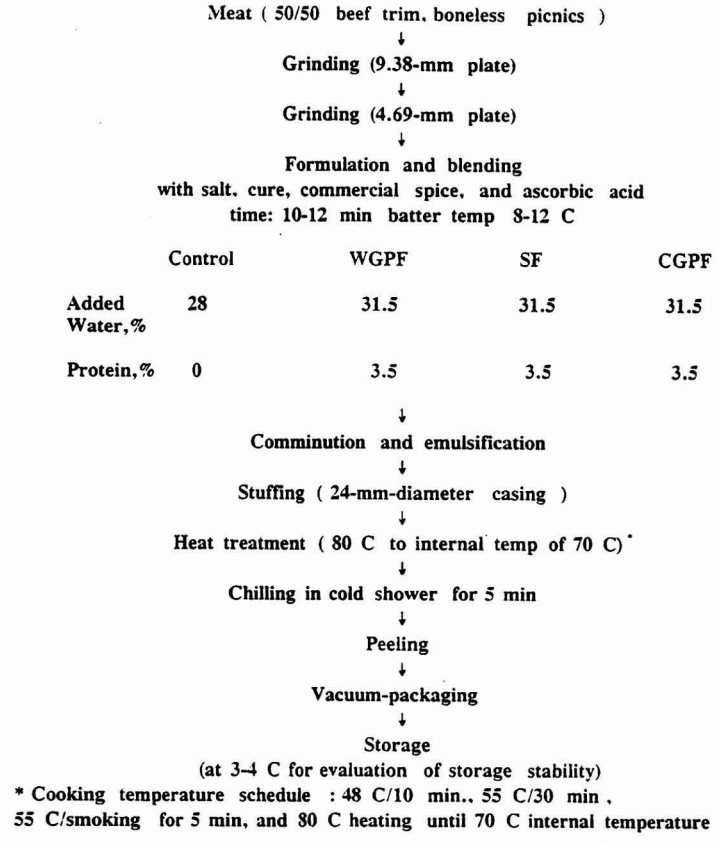


FIG. 1. FLOW CHART OF FRANKFURTER PREPARATION

increased added water at 1% for every 1% additive added. Formulations contained salt (2.84%), Prague powder (0.34%), spice (0.5%) and ascorbic acid (0.1%). A Hobart bowl mixer was used to mix meat with salt and 1/3 of the water for 2 min. Prague powder containing 6.25% sodium nitrite (Griffith laboratory, Alsip, IL) and ascorbic acid were added separately with another 1/3 of the water and finally spice was added with the remaining water, keeping the mixing time constant at 2 min for every addition. For treatments, additives were added at the end, with added water divided equally among each of the four stages of additions and 2 min of mixing during addition of each ingredient. The mix was comminuted through a Mincemaster emulsion mill (Griffith GL-86, Griffith Design and Equipment Co, Chicago, IL) with 1.7 mm plate. The temperature of the batter was controlled not to exceed 8–10C. Batters were stuffed into 24-mm cellulose casings and formed into links of 11 cm. Frankfurters were cooked in a commercial smokehouse (Maurer and Sohne Reichenau,

Germany) with a cooking schedule: 48C/10 min, 55C/30 min, 55C smoking/5 min, and 80C till an internal temperature of 70C. The thermometer was microprocessor controlled and relative humidity of smokehouse was set at 85%. The frankfurters were chilled in a 5 min cold water shower, peeled manually, vacuum packaged in 8 × 12 in. polyethylene-polyvinyl chloride laminated bags (Koch's supplies, Kansas City, MO) and stored in a refrigerator at 3–4C.

## Water Activity ($a_w$)

Water activity ($a_w$) was measured at room temperature using a Decagon CX-1 water activity meter (Decagon, Pullman, WA). Frankfurters randomly selected from four different packages were chopped for 1 min in an Oster blender (Model No 548-41 A). Then 3-g samples were packed into sample cups and placed in the measuring chamber. Direct $a_w$ readings were taken from the digital display. Care was taken while packing into sample cup not to exceed half of the cup.

## Bacteriological Quality

Frankfurters weighing a total of 11 g were removed aseptically, placed into sterile whirl pack bags. A 99 ml portion of sterile saline solution was added, and samples were homogenized for 1 min in a stomacher blender. Five consecutive dilutions of the samples were made by transferring 1 ml of the sample suspension into 10 ml of sterile saline solutions stoppered in dilution bottles. Appropriate dilution aliquotes were drawn with sterile pipet and transferred into sterile disposable petri dishes. Media stored at 45C in a water bath were poured into petri dishes and carefully rotated for uniform distribution.

**Total Psychrotrophic Counts.** A 23.5 g amount of standard plate count agar (Difco Laboratory, Detroit, MI) was suspended in 1 L of distilled water and heated to boiling to dissolve completely. The medium was sterilized in an autoclave at 15 lb pressure and 121C for 15 min and stored in water bath at 45C. Petri dishes were allowed to solidify after pouring, inverted and incubated at 7C for 10 days. Number of colony forming units (CFU) were counted with the aid of an digital colony counter (Manostat Corporation, NY, catalogue #81-520-150). The number of CFUs were multiplied by dilution factor to determine CFUs per gram of sample. CFUs were transformed into corresponding logarithmic numbers for statistical analysis.

**Fecal Coliform Tests.** A 41.5 g amount of violet red bile agar (Difco Laboratory, Detroit. MI) was suspended in 1 L of distilled water and heated to boiling for 2 min to dissolve. It was cooled in a water bath (48C) before use.

After pouring, the samples in petri dishes were allowed to solidify, inverted and incubated at 35C for 24 h. A positive CFU was purplish red colony of 0.55 mm or larger with a halo of 1–2 mm diameter and surrounded by a reddish zone of precipitated bile (Klein and Fung 1976).

### Thiobarbituric Acid (TBA) Values

TBA values were determined using extraction procedure of Witte *et al.* (1970) with a modification (Kuntapanti 1978). Colorimetric absorbance at 529.5 nm was measured using a Perkin Elmer ultraviolet spectrophotometer. A standard solution of 1.1.3.3 tetraethoxypropane(TEP) was prepared by the same method as sample preparation. The TBA values were reported as mg malonaldehyde/kg of sample as obtained from standard curve of TEP.

### Ammonia Nitrogen

Ammonia nitrogen was determined using the colorimetric method (AOAC 1984, 18.027) with minor modification. Colorimetric absorbance (680 nm) readings obtained from a Perkin-Elmer Spectrophotometer (Coleman Model 124D) were converted to quantities of ammonia nitrogen by using a standard curve. Values were reported in mg N/100 g sample.

### Total Volatile Nitrogen (TVN)

A 10 g sample was blended and extracted with 20 ml 7% trichloroacetic acid. The extract was centrifuged and filtered through Whatman No. 1 filter paper. A 5 ml portion of filtrate was used for TVN determination using micro-Kjeldahl distillation (Cobb III *et al.* 1973).

### Statistical Analyses

Statistical analyses systems (SAS 1985) was used for data analyses. A split plot design was employed with treatments on whole plots and storage periods (3, 15, 30 and 45 days) as subplots. General linear model procedure was used. Four replications were performed. Least square means were compared when treatment differences were statistically significant ($P < 0.05$).

# RESULTS AND DISCUSSION

## Water Activity

Frankfurters are classified as highly perishable meat products (Roedel *et al.* 1975). Treatments were not different in their $a_w$, (Table 1). However, all treatments showed an increase in $a_w$ after 45 days of storage (Fig. 2). The increase was significant from 3 days to 15 days ($P < 0.05$), but it was not different from 15 to 45 days. In another study, Vallejo-Cordoba *et al.*(1987) reported a decreased $a_w$ upon addition of protein hydrolysates prepared from SF. Hung and Zayas (1991) reported a nonsignificant increase in $a_w$ during storage of frankfurters containing milk proteins and CGPF. WGPF, SF, and CGPF are multicomponent in nature. While the protein component is primarily responsible for water binding, nonprotein components, ranging from starches to polysaccharides, can also participate in water absorption. Due to this, differences exist in the mechanism of water binding activity among samples. Storage during initial periods (15 days) at 3-4C might have caused certain changes in the water mobility that could increase the $a_w$. However, the increase in mean $a_w$ value from 3 days to 15 days was from 0.977 to 0.983 and may not be of any practical significance as frankfurters are considered highly perishable even before storage.

TABLE 1.
F-VALUES AND SIGNIFICANCE OF STORAGE ATTRIBUTES

| | | F Value | Pr > F |
|---|---|---|---|
| Water activity | | | |
| | Trt | 2.35 | 0.1410 |
| | Storage | 37.38 | 0.0001 |
| | Trt X Storage | 1.49 | 0.1863 |
| Bacterial counts | | | |
| | Trt | 0.78 | 0.5609 |
| | Storage | 906.11 | 0.0001 |
| | Trt X Storage | 0.53 | 0.8849 |
| TVN | | | |
| | Trt | 480.69 | 0.0001 |
| | Storage | 107.19 | 0.0001 |
| | Trt X Storage | 0.78 | 0.6707 |
| Ammonia nitrogen | | | |
| | Trt | 1.91 | 0.2025 |
| | Storage | 52.86 | 0.0001 |
| | Trt X Storage | 0.88 | 0.5719 |
| TBA values | | | |
| | Trt | 10.15 | 0.0008 |
| | Storage | 50.34 | 0.0001 |
| | Trt X Storage | 1.14 | 0.3505 |

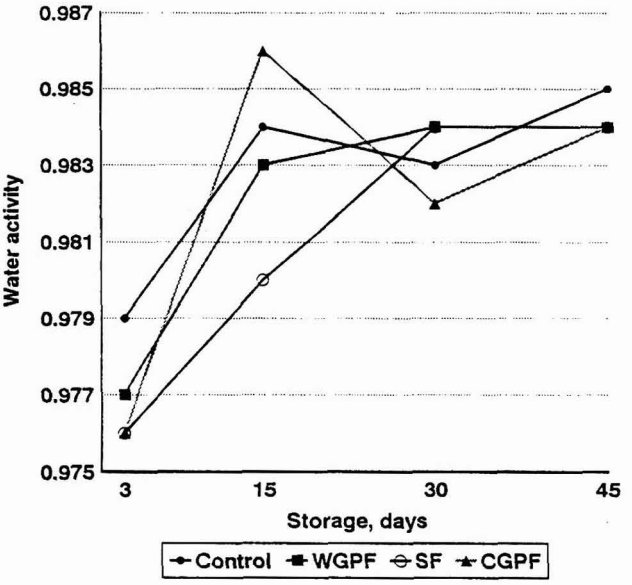


FIG. 2. WATER ACTIVITY OF FRANKFURTERS EXTENDED WITH WGPF, SF, AND CGPF
WGPF = Wheat Germ Protein Flour, SF = Soy Flour, CGPF = Corn Germ Prctein Flour.

## Bacteriological Tests

Psychrotrophs that grow at 1-10C are the major spoilage organisms in CMP during storage at refrigeration temperatures. Treatments were not different in their psychrotrophic counts at any storage periods while significant increase during storage was observed (Table 1). All samples showed an increase in CFU ($P < 0.05$) from 3 days to 45 days of storage (Fig. 3). Hung and Zayas (1991) reported an increase in psychrotrophic counts of CGPF containing frankfurters after 45 days of storage. Interesting reports are observed in the literature on bacterial quality of such products. Sikes and Maxcy (1979) reported that a combination of plant and meat proteins stimulated the growth of certain bacteria during storage, while other investigations also revealed that no such effect was evident due to added protein material (Thomson *et al.* 1978; Foster *et al.* 1978). Microbial quality of CMP extended with nonmeat protein additives depends on a variety of factors such as type and amount of protein additives, initial microbial quality of raw materials, storage conditions, heat treatment, post processing conditions and methods of evaluation. In the present study, nonmeat additives did not influence the bacterial growth in a manner different from controls. No fecal coliforms were observed in any of the samples during

storage. This might suggest: (1) absence of initial contamination, (2) adequate processing temperature, and (3) absence of postprocessing contamination.

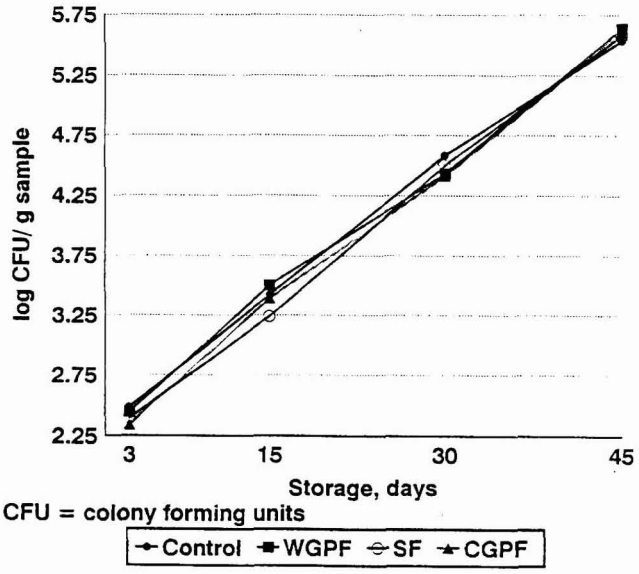


FIG. 3. TOTAL PSYCHROTROPHIC COUNTS OF FRANKFURTERS EXTENDED WITH WGPF, SF, AND CGPF
WGPF = Wheat Germ Protein Flour, SF = Soy Flour, CGPF = Corn Germ Protein Flour.

## Total Volatile Nitrogen

TVN values of samples were affected by treatments and storage periods (Table 1). Samples with SF had higher TVN values than other samples. Increase in TVN upon storage was significant ($P < 0.05$) at every storage period. All samples had higher TVN values after 45 days of storage (Fig. 4). Increased microbial growth and proteolysis during storage might be the major reason for increase in TVN in all the samples. Differences in TVN values among samples might be attributed to the type and structure of proteins and their amino acid composition.

## Ammonia Nitrogen

Treatments did not differ in their ammonia nitrogen content. However, all samples were affected by storage ($P < 0.05$) (Table. 1). The ammonia nitrogen content of all samples ranged from 3.235 mg N/100 g to 6.158 mg N/100 g. All samples had highest ammonia nitrogen at 30 days of storage (Fig. 5) and they

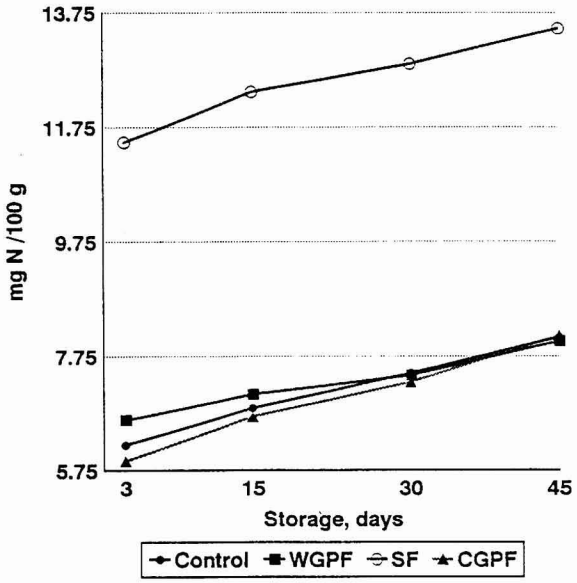


FIG. 4. TOTAL VOLATILE NITROGEN OF FRANKFURTERS EXTENDED WITH WGPF, SF, AND CGPF
WGPF = Wheat Germ Protein Flour, SF = Soy Flour, CGPF = Corn Germ Protein Flour.

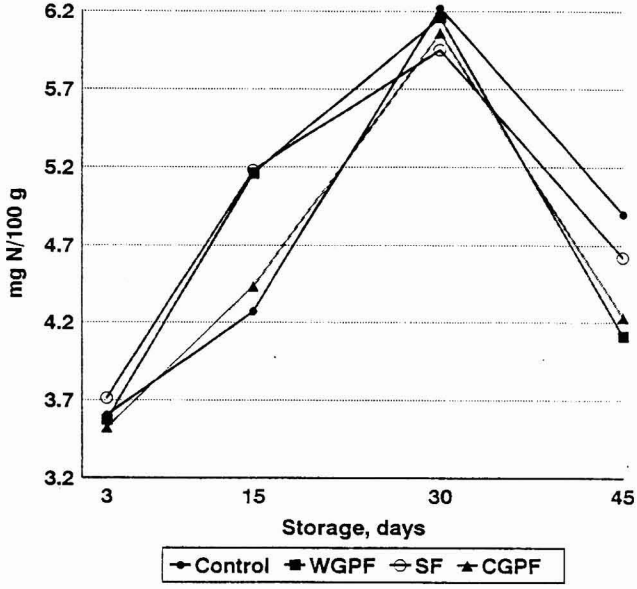


FIG. 5. AMMONIA NITROGEN OF FRANKFURTERS EXTENDED WITH WGPF, SF, AND CGPF
WGPF = Wheat Germ Protein Flour, SF = Soy Flour, CGPF = Corn Germ Protein Flour.

dropped to lower levels after 45 days of storage. During storage, microbial organisms thrive on soluble nitrogenous substances that result from proteolysis (Gill 1976). Free amino acids and nucleotides are some of the nitrogenous compounds that release ammonia upon deamination reaction. A decrease in ammonia nitrogen content of samples from 30 to 45 days of storage might be due to utilization of released ammonia nitrogen by microorganisms or release of ammonia from package due to concentration gradient from inside package and the surroundings. Hence higher concentration of ammonia nitrogen occurred at 30 days of storage than at 45 days of storage.

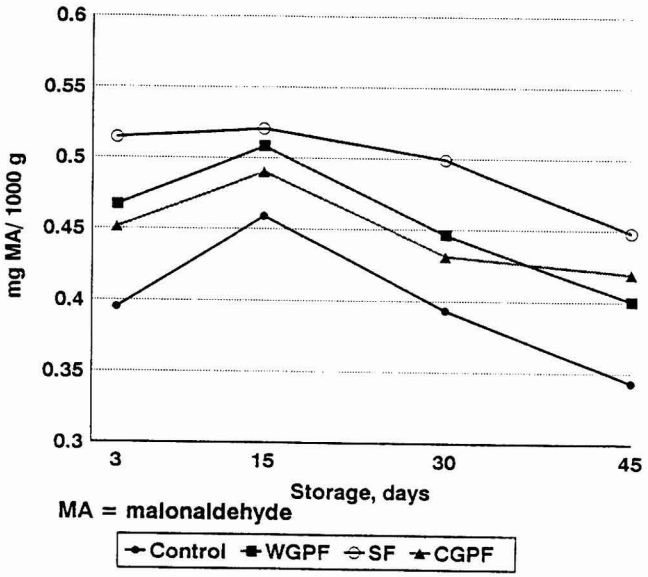


FIG. 6. TBA VALUES OF FRANKFURTERS EXTENDED WITH WGPF, SF, AND CGPF
WGPF = Wheat Germ Protein Flour, SF = Soy Flour, CGPF = Corn Germ Protein Flour.

## TBA Values

TBA values of samples were significantly affected by treatment and storage ($P < 0.05$)(Table 1). Samples with SF had highest TBA values followed by samples with WGPF. Samples with SF and WGPF were not different, while samples with CGPF had lower scores than SF. All treatments had higher TBA values than control samples. TBA values of samples ranged from 0.343 to 0.521 mg malonaldehyde/1000 g. All samples had highest TBA values at 15 days of storage (Fig. 6), while they showed a gradual decrease after that. TBA values

at 30 days of storage were not different from those at 3 days while all samples had lowest TBA values after 45 days. TBA values of meat are well-correlated with oxidative rancidity (Melton 1985). However, malonaldehyde, a highly reactive secondary product of lipid oxidation can react with other components of meat such as peptides, amino acids and amines, forming complexes that are not detected by TBA tests (Witte *et al.* 1970; Tarladgis *et al.* 1960; Gokalp *et al.* 1983). Gray (1978) reported that many compounds of lipid oxidation are capable of interfering with color formation in TBA tests. Sucrose and certain compounds of wood smoke can react with TBA to produce red color identical to that produced by malonaldehyde. This might be a critical factor when performing this test for muscle foods. Buttkus and Rose (1972) concluded that malonaldehyde incorporated into cyclic compounds was more stable during TBA tests than when incorporated into straight chain compounds. Hence, in oxidizing system containing proteins, there could be a competition for malonaldehyde resulting in reduced color development and thus incomplete quantitation of malonaldehyde.

## CONCLUSIONS

Storage stability of frankfurters was affected by incorporation of WGPF, SF, and CGPF. A slight increase in $a_w$ was observed in all samples stored for 15 days and longer. An increase in $a_w$ upon storage over 15 days may not be of practical importance as frankfurters are considered as highly perishable products. Total psychrotrophs increased upon storage up to log 5 CFU/g. Additives did not affect bacterial counts in any manner different from controls. Overall mean values of TVN in samples containing SF and WGPF were higher, while other samples were not different among themselves. Storage at 3–4C significantly increased TVN of all samples. Differences in TVN values might be attributed to structure and composition of proteins. Ammonia nitrogen of samples increased up to 30 days of storage and decreased afterwards. TBA values of samples containing SF were higher than controls and a decreasing trend of TBA was observed after 15 days of storage. This could be due to formation of complexes between malonaldehyde and other breakdown products of meat, such as peptides and amino acids resulting in a decrease in free malonaldehyde present in the sample. TBA values of samples ranged from 0.343 to 0.521 mg malonaldehyde/1000g of sample, which is considered within acceptable limits. Samples containing WGPF were satisfactory in their storage stability. All samples can be considered bacteriologically and chemically acceptable up to 45 days of storage at 3–4C.

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MICHAELS, S.L. 1989. Crossflow microfilters ins and outs. Chem. Eng. *96*, 84–91.

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