Physicochemical Properties of a Time-Temperature Indicator Based on Immobilization of *Aspergillus oryzae* α-Amylase in Polyacrylamide Gel as Affected by Degree of Cross-linking Agent and Salt Content

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ABSTRACT: Currently available time-temperature indicators (TTIs) with favorable thermal inactivation kinetics are physically incompatible with microwave or radio frequency systems. Here, TTIs for microwave and radio frequency food pasteurization processes were developed that have dielectric properties that match target foods. The TTI can be easily recovered from a food and assayed using a rapid, simple colorimetric assay. Enzyme recovery was reduced by 8% but diffusion was retarded by 20% when bisacrylamide content was increased from 3.3% to 5.3%. The specific enzyme activity was highest in 20% polyacrylamide gels containing 2% NaCl. The residual enzyme activity in the TTI following 2 mo of storage at 8 °C exceeded 70%.

Keywords: α-amylase, immobilized enzymes, microwave, radio frequency, pasteurization, food

Introduction

Pasteurized foods are becoming an increasingly important market segment. They meet consumer demands for convenient products and fill important niches in the home-meal replacement and heat-and-serve markets for food service. However, pasteurized food can cause illness if not properly processed. Failing to accurately verify a process increases legal liability (Rasco 1997; Buzby and others 2001). The use of microbial testing to verify process is time-consuming and labor-intensive. Developing a simple and accurate enzyme-based time-temperature indicator (TTI) for determining adequate microbial inactivation within pasteurized foods would be an important development.

Heating a food in hermetically sealed packages can eliminate the possibility of postprocess contamination. In-package pasteurization by novel processes such as microwave and radio frequency (RF) have several advantages, including speed, energy savings, and quicker startup and shutdown times compared with the conventional heating processes (Risman 1991). However, microwave heating and RF heating are not uniform (Schiffmann 1990, 1992; Stanford 1990). The composition, temperature, size, and shape of the food influence the heating rate and heat distribution throughout a packaged food product (Fakhouri and Ramaswamy 1993; Remmen and others 1996).

Process verification and mapping of heat distribution in microwave and RF heating systems are difficult, and current methods are inadequate. Electronic sensors with metallic components are used in conventionally thermally processed foods. However, these sensors cannot be used with microwave and RF systems because they interact with electromagnetic waves, giving misleading data. Current methods using fiber-optic sensors for online temperature measurements are also impractical, expensive, and unsuitable for continuous processes. Also, intrinsic chemical marker methods, such as the qualitative or semi-quantitative assay developed by the US Army Natick Soldier Center for commercially sterile foods (110 °C to 130 °C), cannot be used for pasteurization applications because the chemical markers form slowly under 100 °C, if at all (Kim and Taub 1993).

A suitable TTI system for microwave and RF would match the dielectric properties (dielectric constant [ϵ'] and dielectric loss factor [ϵ'']) to the target food product to ensure that the heating rate of the food product and the TTI are similar. ϵ' indicates the amount of electrical energy that can be stored by the food (Nelson 1994). At lower NaCl concentrations (1%), ϵ' decreases with temperature (Hasted and others 1948). At higher NaCl concentrations (4%), the correlation between ϵ' and temperatures is uncertain (Bengtsson and Risman 1971). In food systems, such as meat treated by microwave radiation (2450 MHz), there is a positive correlation between ϵ' and NaCl content (Nelson 1994).

ϵ'' relates to mechanisms of energy dissipation (Nelson 1994). ϵ'' decreases with temperature at lower NaCl concentrations (1% NaCl) (Hasted and others 1948) and increases with temperature at more than 2% NaCl (Bengtsson and Risman 1971). ϵ' and ϵ'' also vary with frequency, temperature, density, moisture content, homogeneity, particle size, constituent distribution, and physical state of foods (Ohlsson and others 1974; Tong and Lund 1993; Ryynanen 1995).

Currently, no enzyme-based TTI systems have been investigated for microwave or RF pasteurization. The α-amylase from *Bacillus amyloliquefaciens* used stainless steel since the matrix for con-

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ventional thermal processing (Van Loey and others 1997) is incompatible with microwave and RF heating processes. Glass beads provide a possible carrier matrix (de Cordt and others 1992); however, the difficulty of modifying the dielectric properties of a glass-based TTI would limit its use in microwave and RF applications. An effective TTI in a system of immobilized enzyme requires that the TTI possess the following physicochemical characteristics: (1) high recovery of enzyme activity; (2) high retention of enzyme activity over time; (3) a relatively slow diffusion of enzyme from the gel; (4) physical and chemical compatibility with microwave and RF pasteurization processes; and (5) enzymes with z values around 7 °C to 12 °C.

α-amylases (α-1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1) are endo-enzymes, which catalyzed the hydrolysis of α-(1,4)-glycosidic bonds of amylose and amylopectin to a range of maltooligosaccharides (Adams 1991). The enzyme also hydrolyzes α-(1,4)-glycosidic bonds in smaller polyglucans, such as maltodextrin. This enzyme is of particular interest for TTI applications: (1) It is inexpensive and commercially available, and (2) the assay is fast, simple, and inexpensive. Heat labile α-amylases from *Aspergillus oryzae* (Kundu and Das 1970), *Bacillus subtilis* (Yamane and Maruo 1974), and *B. amyloliquefaciens* (Borgia and Campbell 1978), with stability falling within the range of pasteurization temperatures, are candidates for TTI applications. Use and characterization of an enzyme with a somewhat higher thermal resistance than the target pathogen make the development of this TTI realistic.

Polyacrylamide gel has been studied as support matrices for yeast cells (Pundle and others 1988), fungal spores (Bihari and others 1984), and enzymes. Enzymes have been immobilized in polyacrylamide gel including alkaline phosphatase (Pizarro and others 1997), β-galactosidase (Park and Hoffman 1993), riboflavin kinase (Kashchenko and others 1992), lipoxygenase (Pinto and Macias 1996), wheat phytase (Khare and others 1994), and urease (Das and others 1998). Polyacrylamide was chosen here because we could easily alter the physical and chemical properties of the gel to make them compatible with food matrices. Also, it is possible to immobilize enzymes in polyacrylamide with minimal diffusional losses. Enzyme leaking can be minimized by adjusting the ratio of monomer and cross-linking agent (Pizarro and others 1997).

The thermal inactivation properties of these TTIs are somewhat higher than food pathogens, making them appropriate for monitoring microbial inactivation in foods because significant residual activity would remain after a pasteurization process. Z-values for the TTI in test food systems are 10 °C in phosphate buffer, 8.5 °C in minced shrimp, and 7.8 °C in mashed potatoes (Raviyan 2000).

The objectives of this study were to (1) determine the effect of NaCl content and cross-linker percentage on retention of α-amylase activity in the TTI; (2) determine external diffusion of α-amylase from the TTIs; (3) evaluate the enzyme stability in the TTIs during refrigerated storage; and (4) evaluate the possibility of modifying the dielectric properties of the TTIs to match those of target foods for microwave or RF processing.

Materials and Methods

Enzyme

Aspergillus oryzae α-amylase (EC 3.2.1.1) from Sigma Aldrich Co. (St. Louis, Mo., U.S.A.) with a specific activity of 39 units/mg solid or 185 units/mg protein (by Biuret method) was used. One unit will liberate 1.0 mg of maltose from 5 dextrose equivalents (DE) maltodextrin per minute at pH 6.9 at 30 °C. The polyacrylamide and all the other chemicals used were at least analytical-grade (Sigma Aldrich Co.).

Preparation of enzyme solution

For soluble enzyme measurement, 2 units/mL of soluble enzyme was prepared in 25 mL cold 0.05 *M* phosphate buffer pH 7.1 (α-amylase solution). The solution was kept cool in an ice bath and used within 4 h. For immobilization, the concentration of enzyme solution (4.35 mg enzyme protein/mL) was calculated so that 2 unit per piece (about 0.5 × 0.5 × 0.5 cm) of polyacrylamide gel would be recovered following immobilization.

Immobilization procedure

Two polyacrylamide gel systems were used. Both gel systems used the same enzyme concentration (2 units/piece; see method above). One system contained: 66.6% (vol/vol) of solution 1 (20% [wt/vol] acrylamide with 3.3% [wt/vol] bisacrylamide), 31.3% (vol/vol) of α-amylase solution (see above); 2.1% (vol/vol) of 3% (wt/vol) ammonium persulphate; and 30 μL of N,N,N′,N′-tetramethyethylenediamine (TEMED).

The polyacrylamide gel system contained: 66.6% (vol/vol) solution 2 (20% [wt/vol] polyacrylamide gel with 5.3% [wt/vol] bisacrylamide). Other gel constituents were as listed above. The components in solutions 1 and 2 were made up to 100 mL with milliQ water and stored at 4 °C until used.

The immobilized enzyme-containing gel was prepared by blending the enzyme solution into 2 polyacrylamide gel solutions while stirring on a magnetic stir plate with a Teflon®-coated stirring bar for 2 min at low speed. Ammonium persulphate was added, stirred for 1 min, followed by TEMED, and stirred for 0.5 min. The mixture was allowed to polymerize between 2-glass plate separated with flexible rubber tubing around 3 sides (0.5-cm spacing) for 4 h at 4 °C. Gels were stored at 4 °C in Ziploc® plastic bags until used. For routine experiments, the gel was cut into 0.5- × 0.5- × 0.5-cm pieces with a razor blade.

The gels containing NaCl were prepared as described for the 5.3% bisacrylamide gels. Sodium chloride was added to make the polyacrylamide gel solution 1% to 5% (vol/vol) NaCl and stirred for 1 min after the enzyme solution had been added.

Maltodextrin preparation

The substrate was a maltodextrin MALTIN® M040 (Grain Processing Corp. (Muscatine, Wis., U.S.A.). This is a 5DE, bland, white, cold water soluble powder. To block reducing ends, the maltodextrin was treated with sodium borohydride. Five grams of MALTIN M040 was dispersed into 20 mL distilled water and heated for 20 to 30 s to completely dissolve it. This suspension was diluted to 90 mL and cooled in an ice bath. Then a 10 mL cold sodium borohydride solution (0.15 g $NaBH_4$/10 mL distilled water) was added and stirred using a magnetic stirrer for 2 min. The solution was stored in the refrigerator overnight. This stock solution of maltodextrin substrate was stable for several days (Strumeyer 1967).

To prepare the substrate solution for enzyme assay, 0.4 mL of acetone was added drop by drop into 20 mL of the $NaBH_4$-treated MALTIN M040 while shaking. The solution was allowed to stand for 20 min at 22 °C to 25 °C. Then the solution was brought to pH 7.0 with 1 *M* acetic acid. The volume was adjusted to 100 mL with 0.05 *M* phosphate buffer pH 6.9 (Strumeyer 1967). This solution was prepared fresh daily.

Assay of soluble enzyme

The activity of α-amylase (mg maltose/min) was measured spectrophotometrically according to Bernfeld (1955). This procedure is based upon the progressive hydrolysis of the α-1,4 glucosidic bonds in MALTIN M040 at 540 nm. The amount of maltose liberated in the test solution was calculated using reagent-grade maltose as a standard.

For measurement of enzyme activity (30 °C), 1.0 mL of substrate was added to 1.0 mL of pre-equilibrated enzyme solution contained in a 15-mL glass test tube. Enzyme solution was pre-equilibrated to 30 °C. Reaction time was adjusted to obtain an absorbance reading within the linear range of the standard curve. The reaction was terminated by adding 1.0 mL of 0.01 *M* 3,5-dinitrosalicylic acid solution. The color was developed by placing the reaction mixture in a boiling water bath for exactly 5 min, then cooling on ice to 22 °C to 25 °C. Nine milliliters of deionized water was added to each reaction tube before recording the A_{540nm} using Lamda 2 UVOL/VOLIS Spectrophotometer, Perkin-Elmer & Co. (Wellesley, Mass., U.S.A.).

Assay of immobilized enzyme

Three pieces of gel were weighed, added to 3.0 mL of cold extraction buffer (0.05 *M* phosphate buffer, pH 7.1 for gel with 0% and 1% NaCl, and 0.05 *M* phosphate buffer, pH 7.1 with 1.0% NaCl for gel with 2% NaCl), homogenized using a hand-held tissue grinder while on ice, incubated in the refrigeration (4 °C to 6 °C) for 30 min, and centrifuged in a Eppendorf Centrifuge 5415 at 10000 rpm for 4 min at room temperature to remove the precipitated gel. No recoverable enzyme was present in the precipitate. One milliliter of enzyme solution was used for assay using the procedure described previously for soluble enzyme.

Enzyme recovery experiments

Enzyme recovery following immobilization was calculated as the ratio of the activity of the enzyme recovered following immobilization system to the activity of the same units of soluble enzyme. Activity was determined approximately 1 h after polymerization of the polyacrylamide gel was complete. Three experiments were conducted in duplicate.

Enzyme diffusion study

At 22 to 24 h after immobilization, 50 pieces of gel (0.5 × 0.5 × 0.5 cm; approx. weight, 0.12 g/piece) were incubated in a 250-mL beaker containing 200 mL of 0.05 *M* phosphate buffer, pH 7.1. After the desired time of incubation (1 to 6 h) at 20 °C to 22 °C, 3 pieces of gels were collected, and excess buffer solution was removed by blotting with Whatman nr 1 filter paper. The weight of the gel after incubation was measured, and water uptake was calculated. The enzyme activity after incubation was determined as described previously. Three experiments were conducted in duplicate.

Storage stability study

Three samples for each immobilized gel treatment (5.3% bisacrylamide with 0%, 1%, and 2% NaCl) were sealed in a Ziploc plastic bags (total 3 bags/treatment). An open-ended glass tube (0.9-cm dia and 2.75-cm length) containing a roll of sterile cotton soaked with 0.05 *M* phosphate buffer pH 7.0 was placed inside of each bag to prevent gel desiccation. There was no direct contact between the gel and buffer. Samples were stored at 8 °C and enzyme activity determined in duplicate after 0, 5, 10, 12, 20, 40, and 60 d of storage ($N = 3$).

Dielectric properties measurement

Materials. Immobilized enzyme in polyacrylamide gels (5.3% bisacrylamide with 0.00%, 0.50%, 0.75%, 1.00%, 2.00%, and 3% NaCl) were prepared 24 h before dielectric measurements. The gels were placed in Ziploc plastic bags and maintained at 4 °C until tested. The 0.05 *M* phosphate buffer, pH 7.1 with 0.00%, 0.50%, 0.75%, 1.00%, and 2.00% NaCl were used as model foods along with shrimp (*Penaeus monodon*) with an unadjusted pH of 7.1 as a target food. Frozen shrimp (41/50 shrimp/lb), headless with intact exoskeleton in 0.5 lb frozen blocks, were obtained from the local supermarket in Pullman, Wash., U.S.A. and kept at –35 °C. Before the experiment, shrimp samples were placed in plastic bags and thawed under running water at 20 °C. The shrimp were peeled, deveined, placed in a Ziploc plastic bag, and kept in a refrigerator maintained at 4 °C until used.

Methods for dielectric measurement. Dielectric measurements were conducted using the network analyzer (Dielectric probe kit model 85070B, Hewlett Packard, Santa Clara, Calif., U.S.A.). The instrument was calibrated using 3 references: an open circuit, a shorting block, and milliQ water. Dielectric properties at 2 FCC-approved frequencies for food treatments (915 and 2450 MHz) were recorded.

Dielectric measurement of immobilized enzyme. Test samples of enzyme containing polyacrylamide gel (0% to 3% NaCl) were cut into 5.0 × 5.0 cm, placed into a plastic Petri dish, and covered to prevent moisture loss. Before measurement, the dishes were partially immersed in a water bath at a preset temperature (Digi-Bath-™, Laboratory Devices Inc., Fisher Scientific, Pittsburgh, Pa., U.S.A.). Temperature at the center of the gel was measured with a thermocouple (model 600-1020, Barnant Co., Barrington, Ill., U.S.A.). When the sample reached the designated testing temperature (7 °C to 65 °C), the dielectric constant and dielectric loss was measured. A minimum of 10 samples were tested for each treatment.

Dielectric measurement for phosphate buffer solutions. One hundred milliliters of buffer (0.05 *M*) was placed into 250-mL beakers sealed with aluminum foil and a thermocouple inserted into the center of the solution (model 600-1020, Barnant Co.). The beakers were partially immersed in a water bath at a preset temperature until the center reached the designated testing temperature (7 °C to 65 °C) and the dielectric constant and dielectric loss measured immediately. Duplicate experiments were conducted.

Dielectric measurements for shrimp. Test samples of whole peeled shrimp (discussed previously) were placed in the 2-cm outer dia glass tube of 5.8-cm length with a plastic cap to ensure against water or vapor leaks. A small glass tube was inserted through the cap to permit insertion of a thermocouple into the middle of the sample. The tubes were immersed in a water bath at a preset temperature until the center reached the designated testing temperature (7 °C, 25 °C, 40 °C, 55 °C, and 65 °C). The samples were removed from the tubes and placed on an aluminum plate partially immersed in the same water bath (Tanaka and others 1999) and dielectric constant and dielectric loss measurements immediately recorded. A minimum of 15 samples was tested for each treatment.

Statistical analysis

Statistical tests were performed using Minitab® Statistical software, Release 12 (Minitab, Inc., State College Pa., U.S.A., 1997). Means and standard deviation of enzyme activity for diffusion and storage stability studies, dielectric loss, and dielectric constant for dielectric property study were calculated. Recovery of immobilized enzyme was compared with the soluble enzyme using a paired *t*-test (Steele and others 1997). Significance was defined as $P \leq 0.05$.

Results and Discussion

Recovery of enzyme activity

The percentage of enzyme activity retained from gels with either 3.3% or 5.3% of the cross-linking agent N,N′-methylenebisacrylamide, after immobilization was 82.1% ± 5.6% and 74.1% ±

2.0%, respectively. The recoveries are significantly different between the 2 enzyme preparations ($P \leq 0.05$). Acrylamide polymerization is an exothermic reaction, where the amount of heat generated depends significantly upon the level of initiator (ammonium persulphate and TEMED) (Yamamoto and Tatsumi 1986). However, because a constant quantity of initiator was used, and the temperature during polymerization did not increase significantly, this effect was predicted to have little effect on enzyme recovery. The difference in enzyme recovery was primarily due to inactivation through reaction with polymerization reagents and changes to the gel structure (Pizarro and others 1997). Exposure to the cross-linking agent can reduce recovery of enzyme activity as recently reported for the immobilization of urease (Das and others 1998). However, the most probable cause for this loss is a decrease in gel porosity at higher concentrations of cross-linking agent (Baselga and others 1989; Pizarro and others 1997). As the gel porosity decreases, enzyme activity becomes reduced as a result of enzyme-network interactions (Pizarro and others 1997). It is also possible that enzyme molecules become more tightly entrapped in the gel matrix and become more difficult to recover for assay, or alternatively, diffusion of the substrate to the enzyme during assay may have been impeded because of the smaller pore size.

Figure 1 shows that adding NaCl to the polyacrylamide matrix enhances enzyme activity. For 2% added NaCl, a 35% increase for the immobilized enzyme at 5.3% cross-linker concentration. The maximum activity for the free and immobilized enzyme was reached between 2% to 3% NaCl. The chloride ion may assist with enzyme substrate binding to α-amylase (Whitaker 1996).

The level of NaCl in the extracted buffer may also play a role in enzyme recovery. When NaCl (1%) was added to the extraction buffer, losses of activity of about 20% and 10% were observed in gels immobilized with 0% and 1% NaCl, respectively (data not presented). In contrast, the activity of gel immobilized with 2% NaCl remained unchanged under the same extraction conditions. This observation suggests that, besides the effects of the chloride ion, as described previously, optimizing ionic strength of the extraction media may also be important for enzyme recovery.

Enzyme diffusion from gel matrix

For development of a TTI, a slow diffusion rate of enzyme from the immobilization matrix into the food is necessary. Many enzymes have a low diffusion rate from polyacrylamide gel. Practically no leaching of urease from polyacrylamide (10% monomer and 5% cross-linker) was observed over a period of 48 h (Das and others 1998). In general, enzyme activity decreased with incubation time, suggesting leaching from the matrix. The higher cross-linking agent (5.3%) hindered the leaching losses. Despite the lower enzyme recovery for gels with 5.3% cross-linker compared with 3.3%, diffusion of enzyme from gels was retarded in gels with a smaller pore size (Figure 2).

Decreased enzyme diffusion from the gel with higher cross-linker was expected as the network is less porous, so the amount of enzyme retained in the gel increased (Pizarro and others 1997). The difference between the higher and lower level of cross-linker was about 20% over the entire range of incubation times tested. This study shows how a small change in the level of cross-linker may affect enzyme recovery when diffusion of enzyme from the matrix is a concern. Cross-linker percentages higher than 5.3 were not studied because the gels with such high levels were too brittle and had a tendency to crack when handled. However, in food applications for this TTI, the loss of enzyme through diffusion from the gel is low enough to make the application feasible. These experiments were conducted by incubating the gel in a 1:40 (wt/vol) gel:buffer ratio for up to 6 h, a protocol that greatly exceeds what the TTI would be exposed to in a real food system. TTI applications would be for solid foods; therefore, substantially less diffusional loss would be expected to occur. Exposure of the TTIs in a food during processing would be 2 h or less, much shorter than the times used in these experiments.

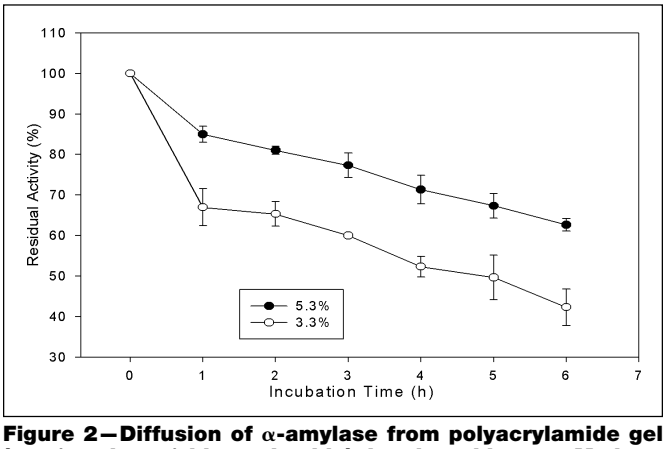

Figure 2—Diffusion of α-amylase from polyacrylamide gel (3.3% and 5.3% bisacrylamide), incubated in 0.05 *M* phosphate buffer, pH 7.1, 22 °C

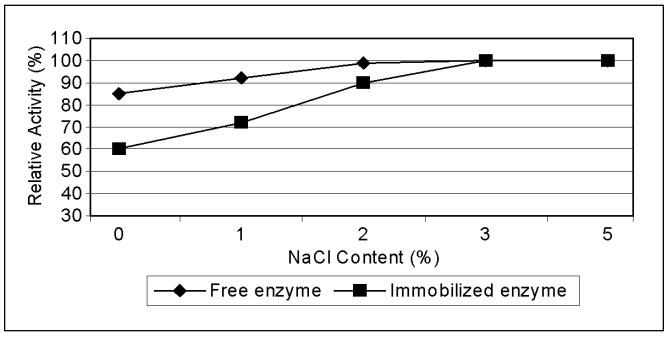

Figure 1—Effect of NaCl content in polyacrylamide gels (5.3% bisacrylamide) on α-amylase activity

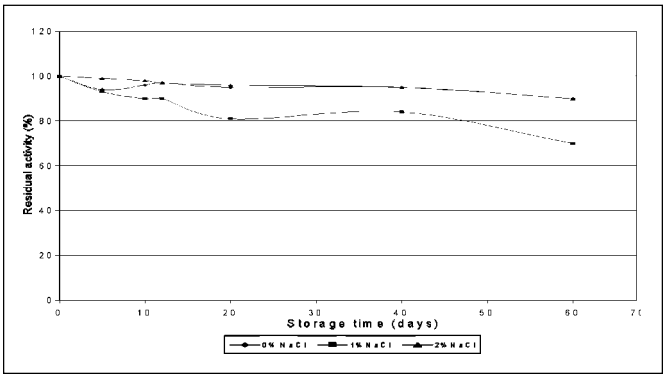

Figure 3—Changes in residual activity of α-amylase immobilized in polyacrylamide gel (5.3% bisacrylamide), stored at 8 °C

Storage stability

Figure 3 shows the changes in enzyme activity as a function of storage time. The residual activity of the immobilized enzyme with 0% and 2% NaCl exceeded 85% after storage for 2 mo at 8 °C; with 1% NaCl, the residual activity exceeded 70%. The significant reduction in the activity of the enzyme immobilized in polyacrylamide with 1% NaCl indicates that enzyme stability is affected by ionic strength and by the differences in variations in ionic strength that may be created in the microenvironment of gel matrix.

Dielectric properties

The dielectric loss (ϵ″) of TTIs (5.3% bisacrylamide) in aqueous solution, a model phosphate buffer system, and in minced shrimp and buffer system at 2450 MHz increased with increasing NaCl concentration (Figure 4 and 5). These finding support previous reports (Ohlsson and Bengtsson 1975; Calay and others 1995). The elevation of ϵ″ results from increased ionic conductivity as the concentration of dissolved salt increases (Mudgett 1986). The ϵ″ of foods with difference levels of NaCl exhibit a different trend with respect to temperature, in this case minced shrimp (*P. monodon*) (Ohlsson and Bengtsson 1975; Calay and others 1995). At lower NaCl concentrations, ϵ″ decreased with increasing temperature. However, ϵ″ increased with temperature at NaCl concentrations of 2% and 3%. The temperature dependence of the ϵ″ at different NaCl concentrations can be explained by the relative effects of dipolar and ionic losses (Ryynanen 1995).

Similar results were observed as an effect of temperature on ϵ″ of gel and buffer with varying salt content at 915 MHz (Figure 6 and 7). At the same temperature, the ϵ″ at 915 was higher than at 2450 MHz. Also, the rate of change in ϵ″ as a function of temperature was more pronounced at 915 MHz, particularly in buffer solutions, than at 2450 MHz. The differences observed for ϵ″ as a function of temperature at 915 and 2450 MHz resulted from a difference in the balance between the conductive loss and the dipolar loss (Calay and others 1995) at these frequencies. The dielectric constant (ϵ′)

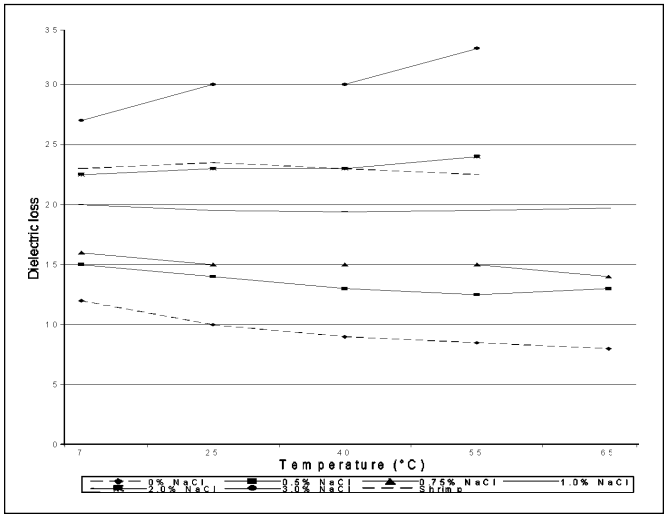

Figure 4—Dielectric loss factor (2450 MHz) for time-temperature indicators (5.3% bisacrylamide) at varying salt concentrations

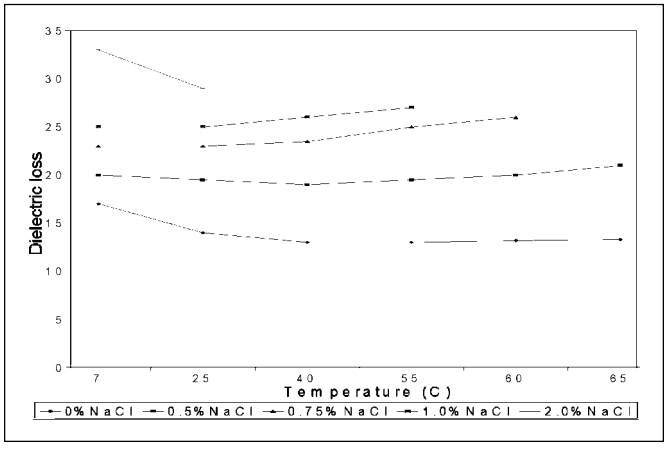

Figure 5—Dielectric loss factor (2450 MHz) of 0.05 *M* phosphate buffer with varying salt content

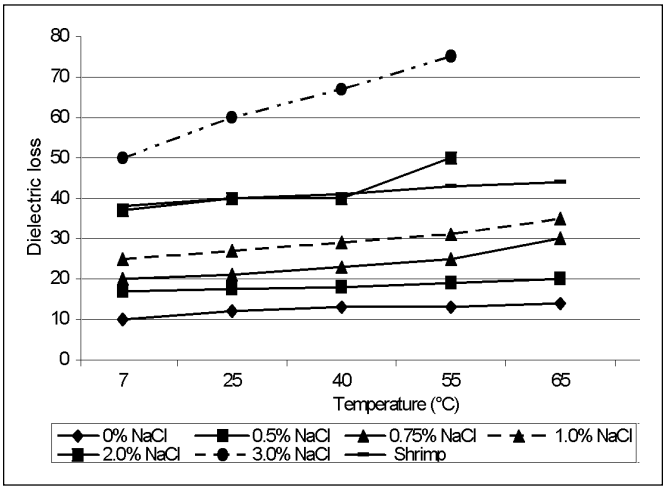

Figure 6—Dielectric loss factor (915 MHz) for time-temperature indicators (5.3% bisacrylamide) at varying salt concentrations

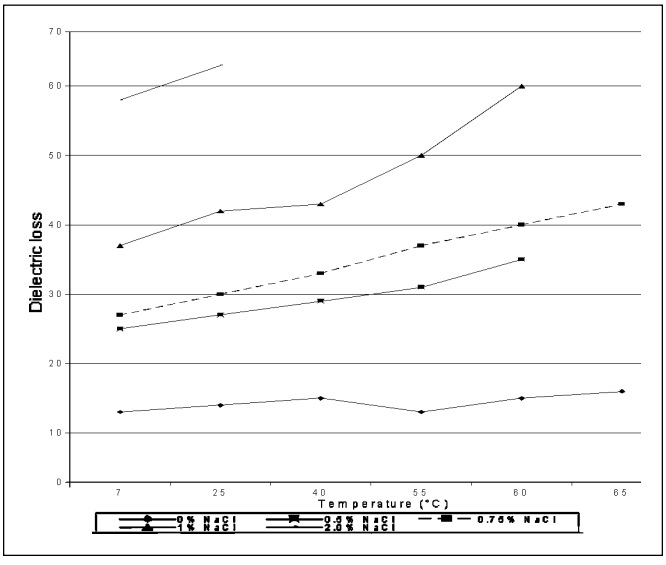

Figure 7—Dielectric loss factor (915 MHz) of 0.05 *M* phosphate buffer of varying salt concentrations

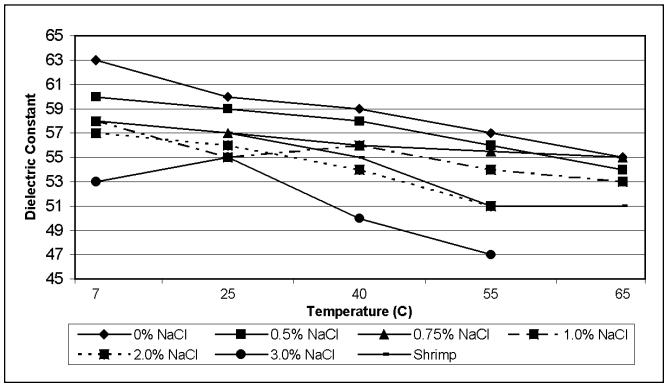

Figure 8—Dielectric constant (2450 MHz) for time-temperature indicators (5.3% bisacrylamide) at varying salt concentrations

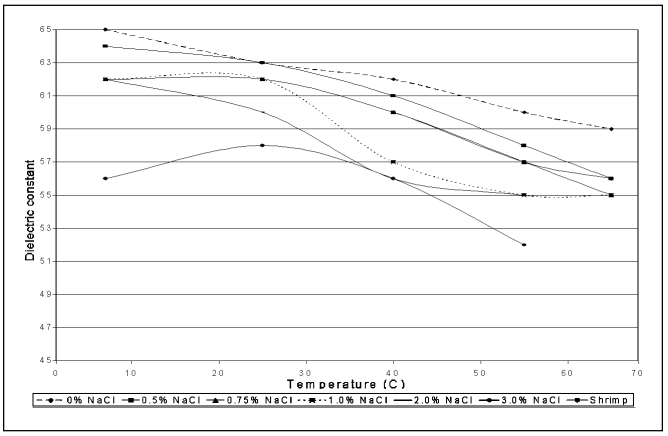

Figure 10—Dielectric constant (915 MHz) for time-temperature indicators (5.3% bisacrylamide) at varying salt concentrations

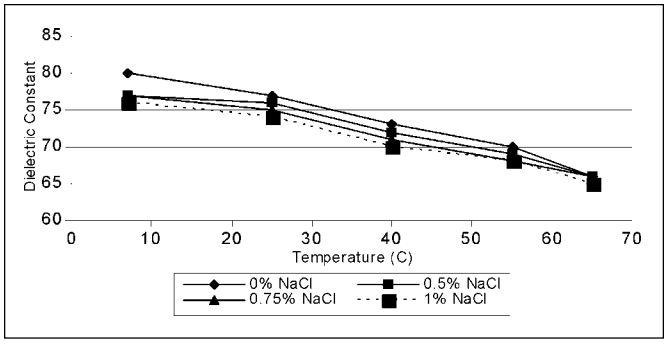

Figure 9—Dielectric constant (2450 MHz) of 0.05 *M* phosphate buffer at varying salt concentrations

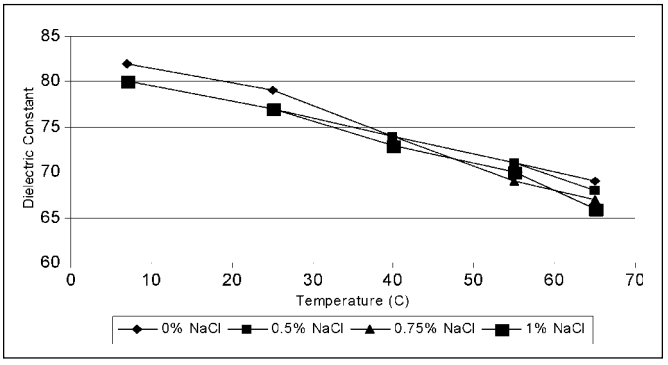

Figure 11—Dielectric constant (915 MHz) of 0.05 *M* phosphate buffer at varying salt concentrations

of the immobilized enzyme gel (TTI) (Figure 8) and buffer solutions (Figure 9) with different NaCl content measured at 2450 MHz and at 915 MHz (Figure 10 [TTI], Figure 11 [buffer]) decreased as salt content and temperature increased.

A similar trend was observed for the immobilized enzyme gels measured at 915 MHz (Figure 10 and 11). However, the effect of changing salt concentration and temperature on ϵ' measured at 2450 MHz was less clear compared with 915 MHz. As reported by other researchers, ϵ' is influenced by many factors including sample composition, temperature, and frequency (Hasted and others 1948; Parkash and Armstrong 1970; Bengtsson and Risman 1971; Ohlsson and Bengtsson 1975; Tong and Lund 1993; Calay and others 1995; Ryynanen 1995).

The ϵ' and ϵ'' yield information about how materials interact with electromagnetic radiation. Knowledge of dielectric properties of the target food sample is important for modifying a TTI system for microwave or RF applications. The NaCl content required to produce TTIs with dielectric properties that match food products are shown in Figure 4 through 7. For example, if shrimp is the target food, dielectric properties can be closely matched with a gel containing 2% NaCl. This study indicates that it is possible to produce TTIs with dielectric properties similar to food.

Conclusions

Enzyme-based TTIs that are physicochemically durable and suitable for the thermal processing of foods, particularly for microwave and RF pasteurization applications, are described here. Enzyme diffusion from the TTI depends upon the level of cross-linking agent, and the enzyme loss observed here under the conditions studied would not limit utility of the TTI. These TTIs could be used in a wide variety of food products and for different pasteurized processes by chemically modifying the dielectric properties of the immobilization matrix to match the food by adding salt or changing the amount of cross-linking agent.

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