Rheological Characteristics of Fibrinogen–Thrombin Solution and Its Effects on Surimi Gels

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ABSTRACT

Gel enhancing effects of fibrinogen-thrombin on surimi were investigated using failure stress and analyzed by mixture design. Gelation time of fibrinogen-thrombin mixture (20:1) gradually decreased as temperature increased from 5°C to 40°C, whereas the G′ and G″ values continued to decrease up to 30°C and then increased at >30°C. Low quality surimi produced higher textural values (stress and strain) when fibrinogen and thrombin mixture (3–5%) had been added. Non-linear stepwise regression model showed no significant interaction effects between surimi and fibrinogen-thrombin mixture. However, the response trace plot showed that failure stress value of surimi gel was increased by the concentration of fibrinogen-thrombin, but decreased by water content.

Key Words: surimi, fibrinogen, thrombin, rheology, mixture design

INTRODUCTION

TRADITIONALLY, THE MANUFACTURE OF MEAT PRODUCTS IS ACCOMPLISHED BY COMMINUTION WITH SALT AND OTHER FUNCTIONAL INGREDIENTS AND THERMAL HEATING. THIS PROCESS DETERMINES THE TEXTURE OF MEAT PRODUCTS THROUGH PROTEIN–PROTEIN OR PROTEIN–WATER INTERACTIONS. HOWEVER, A BINDING SYSTEM IN RESTRUCTURING PORTIONED MEATS WITH NONTHERMAL TREATMENTS (<20°C) HAS BEEN DEVELOPED USING FIBRINOGEN AND THROMBIN EXTRACTED FROM BOVINE PLASMA PROTEINS (FNA FOOD, 1996). THIS MIXTURE CREATES AN ADHESION SYSTEM BASED ON FIBRIN BONDING BETWEEN MOLECULES USING THE ENZYME CONVERSION OF FIBRINOGEN TO FIBRIN.

During blood clot formation, the fibrin stabilizing factor as an inactive precursor is converted to active enzyme forms such as transaminase, transglutaminase or fibrinolysase by the action of thrombin (Bohn, 1972). Fibrin, converted from fibrinogen by thrombin, forms cross-linking in the presence of active enzyme forms and Ca++. Muscle myosin can serve as a cross-linking substrate for active enzyme forms and the heavy chains of myosin carry the most reactive amine incorporation sites (Cohen et al., 1979).

Using a meat binding system for the integrity of fish muscle as well as the red meat portion, low quality muscle protein foods can be upgraded. Its network formation at the lower temperature needs to be rheologically investigated for more food applications. For the optimum mixture of composite foods such as surimi seafood and other comminuted muscle foods, individual ingredient effects on the gel functional properties such as texture, color, and flavor must be determined. The effects of individual ingredients on surimi gels have been extensively studied using carbohydrates (Lee et al., 1992; Yang and Park, 1998) and proteins (Park, 1994). An attempt has been made to develop a least cost linear program for optimization particularly in surimi gels (Lanier, 1988; Yoon et al., 1997a). The interaction effects of surimi and starch also were demonstrated in a mixture with a desirability experimental design (Yoon et al., 1997b). However, relatively few studies have been reported regarding the ingredient interactions in surimi mixtures. Our objectives were (1) to determine the gelation time of fibrinogen-thrombin solution at low temperatures through monitoring changes in elastic and viscous elements and (2) to investigate the effects of surimi and fibrinogen-thrombin on the shear stress of composite gels.

MATERIALS & METHODS

Samples

Low grade (RA) Alaska pollock (Theragra chalcogramma) surimi (American Seafood Co., Seattle, WA) was used to evaluate its gel forming ability in the presence of fibrinogen and thrombin. Frozen fibrinogen and thrombin solutions were obtained from FNA Foods (Calgary, Alberta, Canada). They were thawed in a refrigerator overnight and mixed at 20:1 ratio. In our preliminary study, the 20:1 mixing ratio of fibrinogen to thrombin produced the highest stress value, compared to other mixing ratios (5:1, 10:1, and 15:1).

Measurement of dynamic properties of fibrinogen-thrombin (FT)

Gelation of fibrinogen-thrombin (FT) mixture was evaluated between the cone (diameter = 4.0 cm; angle = 4°) and plate attached to a mechanical dynamic tester (Model CS-50, Bohlin, Cranbury, NJ). Viscoelastic properties such as storage modulus (G’), loss modulus (G’’), and phase angle (tan delta, δ) at test temperature ranges (5–40°C) were measured. A solvent trapper was installed to prevent moisture evaporation during measurement. For the linear viscoelastic region, 1 Pa torque value and 0.1 Hz frequency were selected.

Preparation of surimi gels with setting treatments

Partially thawed surimi tempered at room temperature (~23°C) for ~2h was comminuted in a silent cutter (Stephan Machinery Corp., Columbus, OH) with 3% salt and various concentrations (0, 3, 5%) of FT mixtures. Surimi was adjusted with ice/water to maintain the protein/moisture ratio at 1.5 and used as a control. For the two FT treatments (3, 5%), the FT mixture was added based on the batch size. Total chopping was done for 6 min according to Yoon et al. (1997a). Sample mixture was stuffed into stainless steel tubes (ID = 1.9 cm; L = 17.5 cm). For the control, the sample was set at ~20°C for 0 and 30 min before final heating at 90°C. For the FT treatments, the sample was set at ~20°C for 0, 30, and 60 min before final heating at 90°C. Then heated sample tubes were immediately chilled in ice water.

Measurement of fracture properties of surimi gels

Refrigerated surimi gels were kept at room temperature (~23°C) for 2h to provide an equilibrated temperature. Gels were cut, glued, and milled into dumbbell shape geometry (L = 2.9 cm; end diameter = 1.9 cm; center diameter = 1.0 cm). Fracture test was conducted using Hamann torsion geltometer (Gel Consultants, Raleigh, NC) to measure fracture shear stress and shear strain (Yoon et al., 1997a).

Experimental design, modeling, and trace plot

Experimental design, data analysis, and trace plots were accom-
plished using commercial software, Design Expert (Stat-Easy Co., Minneapolis, MN). The minimum and maximum proportions for the mixture design were set at surimi 80–88%, fibrinogen–thrombin (FT) 0–10%, and added water 3–13%. Fixed levels of salt (3%) were equally applied to all treatments. Upper and lower limits of moisture content in surimi-FT mixture were set at 75–76% in order to fix each vertex. Moisture content was determined (AOAC, 1990). Modified distance-based design (Snee, 1979) was selected to determine a combined mixture of all components. Quadratic experimental design was used to allocate experimental points. Quadratic models with a canonical form and step-wise regression were used for F-tests (Yoon et al., 1997a,b). Trace plots for selected models were drawn in order to show all components in the mixture using Piepel’s direction (Cornell, 1990).

RESULTS & DISCUSSION

Viscoelastic values of fibrinogen–thrombin mixture

Viscoelastic properties such as storage modulus (\(G'\)), loss modulus (\(G''\)), and tan delta (\(\delta\)) of fibrinogen–thrombin mixture (20:1) were changed as a function of temperature (Fig. 1 to 4). At the beginning of reaction time (up to 630s) during holding at \(5^\circ\)C, the magnitude of \(G''\) was much larger than \(G'\), but this order was reversed as the reaction time was extended (Fig. 1). The cross point of \(G'\) and \(G''\) is called gel point of the cross-linking polymer and is an indication of a transition from viscoelastic liquid to solid state (Winter and Chambon, 1986; Muller et al., 1991; Hsieh et al., 1993). After the gel point, all protein aggregates are instantaneously bound together into one continuous molecular structure (Hsieh and Regenstein, 1992). As the holding temperature increased from \(5^\circ\)C to \(15^\circ\)C (Fig. 2), \(25^\circ\)C (Fig. 3), and \(35^\circ\)C (Fig. 4), these sol–gel chemical transition points of fibrinogen–thrombin mixture changed from 630s to 420s, 170s, and 120s, respectively. This trend indicated that the gelation of FT was temperature-dependent more rapidly at lower temperatures (5 to \(15^\circ\)C). The phase shift or sol to gel transition as indicated by log delta (Hamann, 1992) occurred earlier, as the holding temperatures increased from \(5^\circ\)C to \(35^\circ\)C.

Overall changes in gelation time and modulus values at \(G' = G''\) were compared as a function of temperature (Fig. 5). As the holding temperature increased from \(5^\circ\)C to \(40^\circ\)C, gelation time decreased rapidly up to \(20^\circ\)C and then slowly decreased between \(20^\circ\)C and \(40^\circ\)C. However, the modulus value at the gel point decreased from 82 Pa to 14 Pa as the temperature increased from \(5^\circ\)C to \(30^\circ\)C, then increased...
its value at higher temperatures (>30°C) (Fig. 5). This indicated that the fibrinogen–thrombin mixture formed stronger gels at the lower temperatures (5–20°C) and its gelation time was highly temperature-dependent.

**Fracture properties of gels made of FT and surimi**

Effects of setting treatment and concentration of fibrinogen–thrombin mixture were evaluated on the fracture shear stress and shear strain of surimi gels (Table 1). Comparing surimi gels with and without setting, set gels produced the higher shear stress (52.15 kPa) and higher shear strain (1.71) indicating harder and more deformable gels. Addition of 3% fibrinogen–thrombin to surimi resulted in much higher fracture properties (61.77 kPa of stress and 1.98 of strain). This result indicated that the fibrinogen–thrombin mixture effectively contributed to the enhancement of the gel network. As setting time increased from 30 to 60 min, these gel fracture properties gradually increased up to 76.2 kPa for stress and 2.18 for strain. Addition of 5% FT mixture to surimi gels improved gel fracture properties when setting time was 30 min, but longer setting time (60 min) did not increase shear stress and strain values (Table 1). This likely indicated the concentration dependency of thrombin in a coagulation process (Griffin, 1995; Gibbs et al., 1995). The addition of FT mixture to surimi gel enhanced gel fracture properties, but its effectiveness appeared to be dependent on the optimum concentration of thrombin. This is supported by our preliminary tests, which exhibited the highest stress value with the 20:1 mixing ratio (fibrinogen: thrombin) compared to the other ratios (5:1, 10:1, 15:1).

**Nonlinear mixture model and trace plot**

The experimental points in terms of values to determine the ingredient effects were selected with software, Design Expert (Table 2). Each mixing ratio of surimi to fibrinogen–thrombin mixture was determined from modified distance-based design. A total of 13 experimental points were determined including two replicates. Shear stress values ranged between 41.44 and 56.86 kPa, depending on the formulation. Shear strain values did not fit the linear or nonlinear model.

A nonlinear model built by step-wise regression was evaluated by F-test (Table 3). The non-linear model showed a low probability (0.02) for shear stress response, which is acceptable for model validity (Cornell, 1990). According to the lower probability and higher lack of fit value, the nonlinear model was accepted for the shear stress response and the effect of each component and interaction effect in the fibrinogen–thrombin-surimi mixture was expressed by a nonlinear canonical form (Table 4). The constraint coefficient (64.86) of fibrinogen–thrombin did not show any interaction effects with surimi or water on shear stress. The interaction terms in the quadratic model implied that the shear stress in the mixture was affected not only by the interactions between surimi and water but also by individual components.

On the basis of the nonlinear model, a trace plot was generated to show the response trend of each component in the mixture (Fig. 6). A reference blend was set at a centroid of vertices of the mixture. The trace plot showed that shear stress was positively affected by surimi, while the addition of water could negatively affect shear stress. The addition of fibrinogen–thrombin influenced the shear stress value depending on the concentration, but generally increased the shear stress value of a composite surimi gel.

**CONCLUSIONS**

A FIBRINOGEN–THROMBIN MIXTURE WAS VERY REACTIVE IN FORMING a strong structure at lower temperatures and very sensitive to
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setting time and temperature. Mixing fibrinogen–thrombin into low grade surimi enhanced gel fracture properties such as shear stress and shear strain. This indicates that fibrinogen–thrombin could possibly be used to upgrade low quality surimi. A trace plot could be used to demonstrate the response trends of individual ingredients on gel strength.

![Trace plot of each component affecting shear stress](image)

**Fig. 6 Trace plot of each component affecting shear stress. A-A: Surimi; B-B: Fibrinogen–thrombin; C-C: Water.**

**REFERENCES**


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