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Simultaneous Application of Hydrostatic Pressure and Microbial Transglutaminase as Pretreatment to Improve the Physicochemical Properties of Heat-induced Gels from Tilapia Surimi Paste

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ABSTRACT

Tilapia pastes were subjected to pretreatment using a combination of hydrostatic pressure processing (HPP) (0.1 to 300 MPa/25°C/60 min) and simultaneous addition of microbial transglutaminase (MTGase) (0.044 unit/g of paste), which was followed by cooking at 90°C for 20 min, to investigate the changes in the physicochemical properties of heat-induced gels. The pretreatment with MTGase at 300 MPa produced a gel having the strongest breaking force and strain compared with all the other treatments in the study; the gel strength was 3.5 times higher than that of the gel obtained by the 300 MPa treatment without adding MTGase. The combined use of MTGase and HPP for pretreatment could also improve the water-holding capacity of the gel, the value of which ranged from 63.12 to 89.34%. Adding MTGase also could decrease protein solubility under different pressure treatments. Thus, pretreatment of the sample is a better way to improve the gel-forming ability of tilapia surimi.

Key words: gelation properties, hydrostatic pressure, microbial transglutaminase, tilapia surimi

INTRODUCTION

The application of high-pressure technology as a food-processing tool is gaining popularity. High-pressure processing HPP has been shown to induce gel formation in egg white, egg yolk, rabbit meat, suspension of soy protein, and milkfish actomyosin[1-2]. High-pressure technology can induce gelation of proteins in fish muscle to yield new products or analogues of existing products, in which color, flavor, and nutritional value are only minimally affected[3]. Compared with heat-induced gels, pressure-induced gels of fish proteins are softer, more elastic, and with less change in their native color and flavor[1]. Bluefish gels formed by pressure are more translucent, softer, and more digestible than those formed by cooking at 90°C for 20 min[4].

The mechanical properties of surimi can be enhanced by incubation at temperatures below 40°C. This phenomenon, reflecting the protein crosslinking induced by an endogenous calcium-dependent transglutaminase (TGase), is called setting or suwari[5]. Endogenous TGase in walleye pollack surimi has been reported to be relatively pressure-sensitive, and therefore cannot be applied in high-pressure gelation[6]. However, Lee and Park[7] indicated that microbial transglutaminase (MTGase) is pressure-resistant compared to TGase. MTGase, dissolved in buffer solution, showed a remarkable stability toward high-pressure treatment above 400 MPa at 60°C[8]. Seguro, Kumazawa, Ohtsuka, Toiguchi, and Motoki[9] reported that breaking strain was reached highest with 0.03% MTGase under 45°C setting for 30 min; however, there may be a decrease in breaking strain due to excess formation of ε-(γ-glutamyl)-lysine isopeptide when the concentration of MTGase is too high. In a previous study, adding MTGase addition (0.044-0.220 unit/g of paste) could reasonably improve the properties and quality of tilapia surimi gel[10].

Based on previous studies[11-12], the texture and rheological model test could extrapolate the gelation properties which were largely influenced by HPP and MTGase. Although many literatures can be found on the separate application of MTGase and high hydrostatic pressure to various food-processing methods, there is still a glaring

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lack of knowledge on their combined effects[13].

In this study, the simultaneous application of MTGase (0.044 unit/g of paste) and high pressure (0.1~300 MPa; 60 min/25°C), as pretreatment was investigated, to improve the physicochemical properties of heat-induced gels obtained from tilapia paste.

MATERIALS AND METHODS

I. *Materials and Chemicals*

Live tilapia (*Orechromis niloticus*), weighing approximately 500~600 g, were purchased from a local retail market and kept on ice before sample preparation. The fish were filleted immediately and minced in a chopper with a hole size of 5 mm. MTGase ($ACTIVA_{TM}$ WM) was obtained from Ajinomoto Co., Inc. Tokyo, Japan, which had an activity of 44.25 unit/g of a maltodextrin carrier. All other chemicals used in the investigation were of analytical grade and obtained from commercial sources.

II. *Surimi Paste Preparation*

Surimi formulations consisted of 80% moisture, 2% NaCl, and 0.044 unit/g MTGase (0.1%), were minced into a paste in a mortar at 25°C. Before HPP treatment, the surimi was stuffed into flexible plastic casings (Krehalon Soplaril, Barcelona, Spain) of 40 μm thickness and 3.5 cm diameter.

III. *High Pressure and Thermal Treatments*

A high-pressure apparatus (CIP UNIT, No.471-046, Mitsubishi Heavy Industries Ltd., Hiroshima, Japan) with an oil pressure generator and a compressing vessel of flat-bottomed cylindrical interior was used for the treatment. The temperature of the vessel during compression in this experiment was controlled at 25°C. Samples were treated at 0.1 to 300 MPa for 60 min. The quality of the pressurized meat was assayed as described below.

Subsequent to the high-pressure treatment, the gels formed were cooked at 90°C for 20 min and then incubated in ice-water for 15 min. The surimi paste was treated with 0.044 unit/g MTGase (0.1%) at 0.1 to 300 MPa for 60 min. The control was without high-pressure or MTGase treatment.

IV. *Texture Analysis*

The texture analysis of the sample was carried out using a CR-200 D rheometer (Sun Scientific Co. Ltd., Japan) with a ball-type plunger of 5 mm diameter, and the gel strength was measured with a table speed of 200 mm/min. The gel strength was expressed as the product of the breaking force (g) and breaking strain (mm), g × mm[14].

V. *Determination of MTGase Activity Measurement*

MTGase activity was measured according to Folk[15]. The reaction mixture containing 100 μL of enzyme, 700 μL of 0.1 M tris–acetate buffer (pH 6.0), 150 μL of 0.1 M carbobenzoxyl-L-glutaminyl-glycine and 50 μL of 2.0 M hydroxylamine was incubated at 37°C for 10 min. The reaction was subsequently stopped by adding an equal volume (500 μL) of 15% trichloroacetic acid solution containing 5% $FeCl_3$. After centrifugation for 5 min at 6000 × g, the supernatant was collected and the absorbance at 525 nm was measured. The calibration was carried out using L-glutamic acid–γ-monohydroxamic acid as standard. One unit of MTGase activity was defined as the amount of enzyme that catalyzes the formation of 1 μM of hydroxamic acid in a 1 min reaction at 37°C. The TGase activity of tilapia surimi in this study was 0.022 unit/g of meat.

VI. *Dynamic Rheological Analysis*

A rheostress RS-100 (HAAKE, Germany) was equipped with a parallel plate measuring tool (diameter = 6 cm, gap-size setting = 2 mm). Oscillatory measurements (25°C) at a constant shear stress of 80 Pa over the 0.1 to 100 Hz frequency range of 0.1 to 100 Hz was used to determine the storage modulus (G'), loss modulus (G"), and loss tangent (tan δ = G"/G'). The frequency was situated in the centre of the linear viscoelastic region, in order to compare different treatments. To facilitate the comparison of samples with various treatments, the reference frequency of 1 Hz was adopted[16]. The maximum shear stain of 0.04 was set to lie within the linear viscoelastic regime. G' and G" represented the elastic and viscous components, respectively. All measurements were conducted in triplicate.

VII. *Determination of Protein Solubility*

Protein solubility was determined as described by Benjakul *et al.*[17] with some modifications. The sample (1 g) was homogenized for 1 min in 20 mL of 20 mM Tris-HCl, pH 8.0, containing 1% SDS, 8 M urea, and 2% (v/v) β-mercaptoethanol. The mixtures were placed in plastic-capped nalgene tubes, flushed with nitrogen after filling, heated to 100°C for 5 min to inactivate proteases, and stirred continuously overnight at room temperature. Samples were subsequently centrifuged at 20,000 × g for 30 min. Proteins in the supernatant were precipitated by the addition of 50% (w/v) TCA to obtain a final concentration of 10%. The mixture was kept at 4°C and then centrifuged at 20,000 × g for 30 min. The precipitate was washed with 10% TCA and solubilized in 0.5 M NaOH. The protein content was measured by the Biuret test[18].

VIII. *Determination of Water-Holding Capacity*

The water-holding capacity (WHC) was measured by

a method modified from Roussel and Cheftel[19]. Surimi gel were placed in tubes and centrifuged (Sorvall RC-5B; Du Pont Instruments, Wilmington, Del.) at 3000 × g for 20 min. WHC was determined using the relationship:

$$WHC = \frac{P_0 - P_F}{P_F} \times 100\%$$

P_0 is the initial sample weight (before centrifugation) and P_F is the final sample weight (after centrifugation).

IX. *Statistical Analysis*

The Statistical Analysis System (SAS Inst. Inc., Cary, N.C., U.S.A.) was adopted to carry out data analysis and statistical computations for the analysis of variance (ANOVA) and for the Duncan's test. Significance of differences was defined at $p < 0.05$. The differences among treatments were verified by their least significant difference.

RESULTS AND DISCUSSION

I. *Effects of High-Pressure Treatment on the MTGase Activity*

Nonaka *et al*.[20] reported that MTGase activity could be well preserved after high-pressure treatment. Lauber *et al*.[21] also reported that MTGase activity was insignificantly affected by high-pressure treatment under 200 MPa at 20°C, though treatment at pressures above 400 MPa would eventually denature MTGase. Therefore, a final treatment pressure below 300 MPa for up to 120 min at 4°C was chosen for this study. The sample containing MTGase that was obtained under 0.1 MPa treatment, was considered 100% active. The effect of high-pressure treatment (0.1 to 300 MPa) for 0 to 120 min on MTGase activity was shown in Figure 1. After treatment at 50, 100, 200, and 300 MPa for 120 min, the relative MTGase activity decreased to 77.12, 75.62, 75.21, and 75.01%, respectively. It thus is obvious that MTGase could substantially retain its activity after high-pressure treatment. However, the difference in the degree of inactivation was observed because the activity of the sample depends not only on the reaction conditions (pressure, time, and temperature) and system (model and food system) but also on the purity and origin of the enzyme[22]. After treatment at 0.1, 50 and 100 MPa for 120 min, we found significant decrease in MTGase activity was observed. However, at 200 and 300 MPa the decreased in MTGase activity were unsignificantly different. It is concluded that high pressure treatment can influence remarkably on the enzyme activity.

II. *The Effects of MTGase and High-Pressure Treatment on Surimi Gelation Properties*

The breaking force, breaking strain, and strength of the surimi gel were measured to determine the effect of combining MTGase addition with high-pressure treatment on the texture of surimi gel (Figure 2). The breaking-force value of surimi gel tended to increase with pressure after cooking at 90°C for 20 min. When MTGase was added to the surimi paste, this tendency was even more evident. Compared with the control, the breaking-force value of surimi gel increased from 80.0 to 517.5 g when the samples were pretreated at 300 MPa for 60 min at 25°C with MTGase. The breaking-force value of surimi gel with MTGase was higher than that without MTGase at the same treatment pressure. However, different pressure treatments cause the breaking-strain values to be irregular, regardless of MTGase addition. The best strength of the surimi gel in this study was 3067.3 (g × mm), which resulted from the pretreatment with MTGase under 300 MPa for 60 min at 25°C. The gel strength was 3.5 times higher than that of the gel obtained by 300 MPa treatment without MTGase. However, at other pressure treatment (50 to 200 MPa), the enhancement was not significant as at 300 MPa. The heat-induced gel obtained from surimi paste by pressure pretreatment (0.1 to 300 MPa, 60 min, 25°C) and subsequent cooking at 90°C for 20 min showed an increased strength; however, the simultaneous addition of MTGase produced a significant and efficient increase in gel strength. Ashie and Lanier[23] reported that high-pressure treatment renders protein substrates more amenable to MTGase catalysis and it might furthermore enhance the MTGase activity. These results suggested that treatment of high-pressure together with MTGase could enhance the protein structure, and improve the gelation properties.

III. *Determination of Dynamic Rheological Properties*

Viscoelastic bodies, such as sols and gels, show

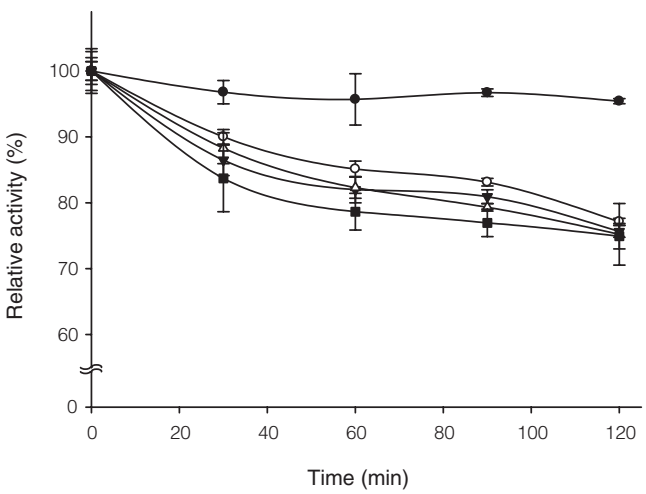

Figure 1. The variation of MTGase activity during different pressure treatments ranging from 0.1~300 MPa for 0 to 120 min at 4°C. (●) 0.1 MPa; (○) 50 MPa; (△) 100 MPa; (▼) 200 MPa; and (■) 300 MPa.

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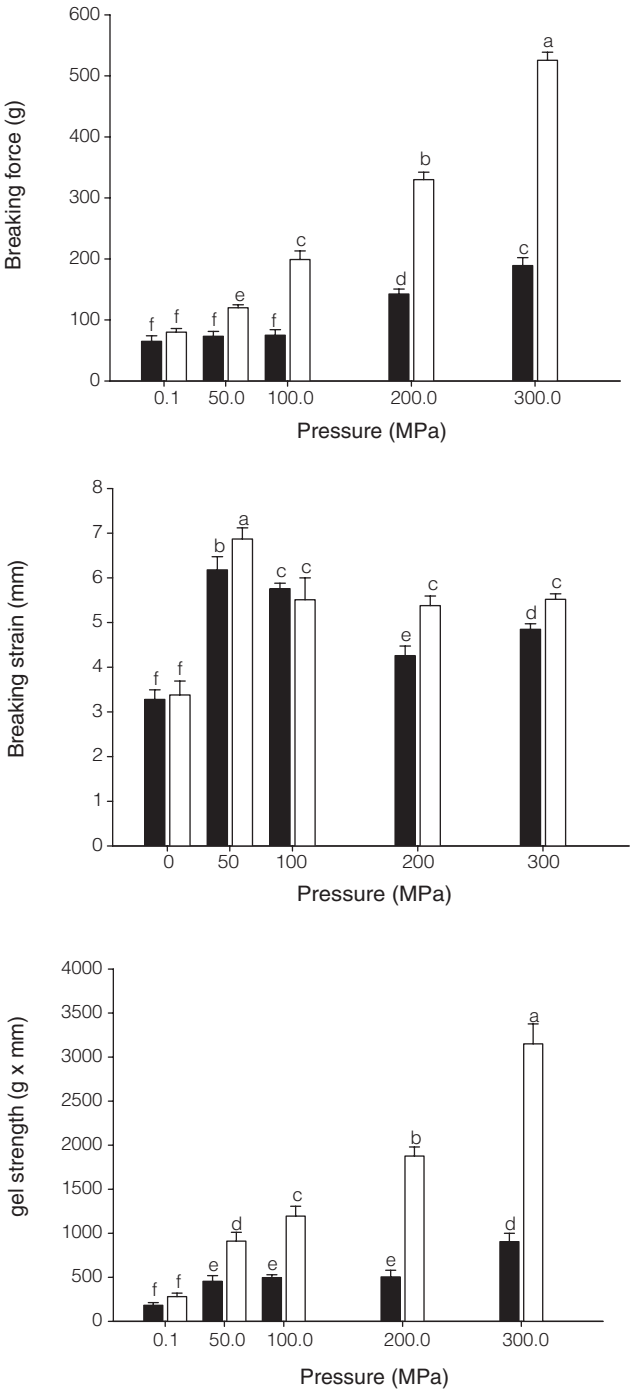

Figure 2. Changes in gel-forming abilites of the surimi gel from tilapia paste, prepared under pressures of 0.1~300 MPa for 60 min and subsequently cooked at 90°C for 20 min. (■) without MTGase and (□)with MTGase. Different letters indicate differences ($p < 0.05$) between the various treatments (columns).

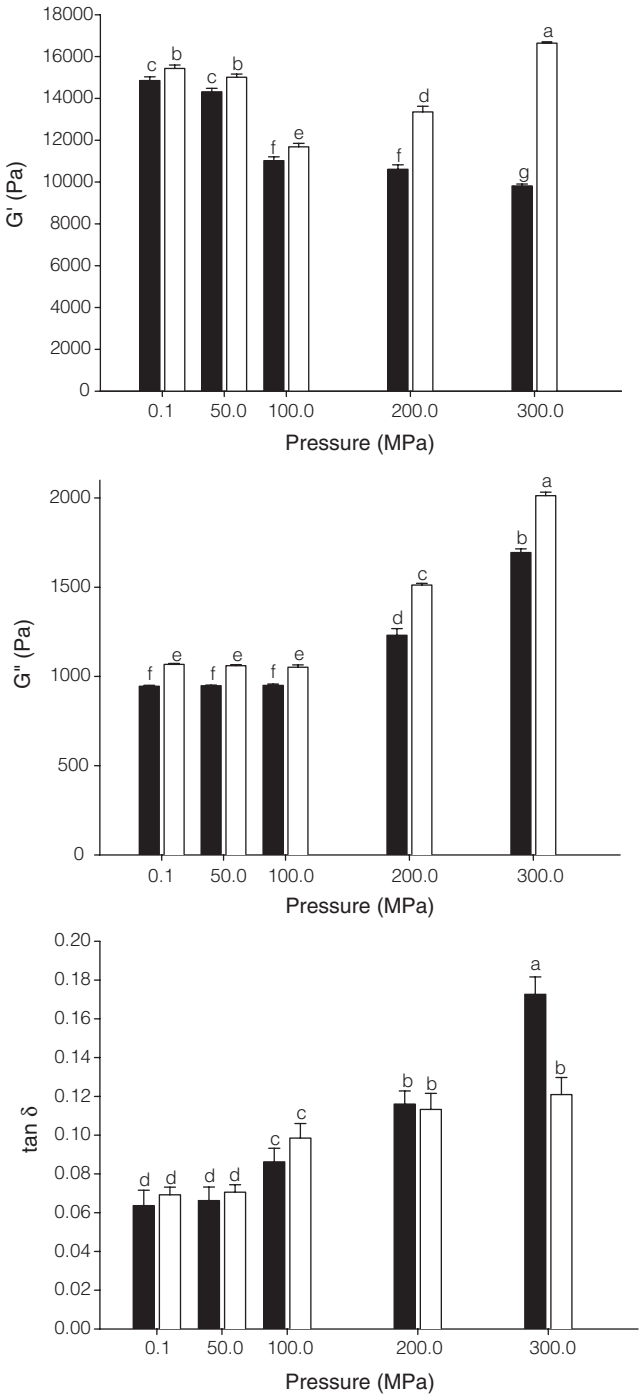

Figure 3. Changes in the rheological properties of surimi gel from tilapia paste prepared under pressures of 0.1~300 MPa for 60 min and subsequent cooking at 90°C for 20 min. (■) without MTGase and (□) with MTGase. Different letters indicate differences ($p < 0.05$) between the various treatments (columns).

both elastic deformation and viscous flow when the stress is applied[24]. Autio *et al.*[25] reported that gels formed by high-pressure pretreatment are rigid and elastic. A comparison of the dynamic rheological properties of surimi gel processed by MTGase and high-pressure treatment is shown in Figure 3. In this study, the G' values of the gels decreased during the high-pressure and MTGase treatments (0.1 to 300 MPa). At 300 MPa, gels with MTGase showed the highest G' value (16641 Pa). The G' and G" values of the gels with MTGase were higher than those without MTGase. The G" values of the gels increased as the pressure was increased from 200

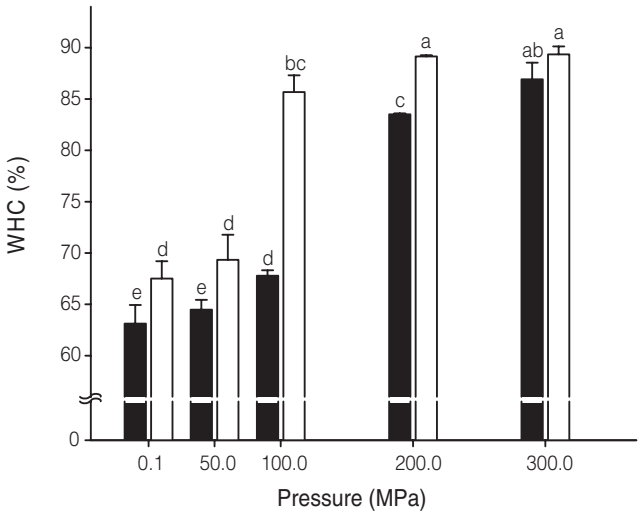

Figure 4. Changes in water-holding capacity of surimi gel from tilapia paste prepared by treating the gel at 0.1~300 MPa for 60 min and subsequent cooking at 90°C for 20 min. (■) without MTGase and (□) with MTGase. Different letters indicate differences ($p < 0.05$) between the various treatments (columns).

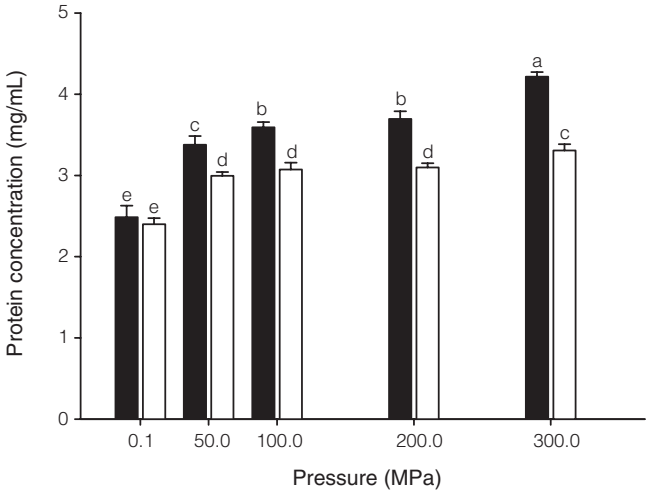

Figure 5. Changes in the solubilites of proteins in the surimi gel obtained from tilapia paste by treating at 0.1~300 MPa for 60 min and subsequent cooking at 90°C for 20 min. (■) without MTGase and (□) with MTGase. Different letters indicate differences ($p < 0.05$) between the various treatments (columns).

to 300 MPa. At 300 MPa, the gels with MTGase showed the highest G" values (2013 Pa). The results showed that the added MTGase induced the surimi gel to form both elastic and viscous gels after pressure treatment. Tan δ values of the gels obtained by all the above treatments were less than 1, which indicated that the gels contained more elastic components than viscous components. The simultaneous application of MTGase and high pressure induced gel formation through hydrophobic interactions and glutamate-lysine bonds[20, 26-27]. Angsupanich and Ledward[28] also indicated that high-pressure treatment at 200 MPa could induce polymerization of surimi proteins, resulting in the formation of more intramolecular hydrogen bonds and hydrophobic interactions.

IV. *Determination of WHC*

WHC of the surimi gels is crucial both in commercial and consumer acceptance[29]. MTGase can induce the formation of interprotein glycoside bonds, which consequently increase water retention[30]. As shown in Figure 4, combining MTGase and high-pressure treatments increases the WHC of tilapia surimi gel. Under high-pressure treatment at 0.1 to 300 MPa, the WHC of the surimi gel containing MTGase was increased from 67.51 to 89.34% and that of the gel without MTGase was increased from 63.12 to 86.91%. When the treatment pressure increased to 200 MPa and 300 MPa, the surimi gel containing MTGase showed a better WHC, which was 1.32 times higher than the control. Other reports also indicated that MTGase enhanced the WHC of proteins either by increasing their ability to swell and to take up water or by improving their ability to form a gelling network[31].

V. *Determination of Protein Solubility*

Hsu and Jao[32] have reported an increased protein solubility of surimi gel after an increased pretreatment pressure. As shown in Figure 5, when the pressure was raised from 0.1 to 300 MPa, the solubility of proteins in the surimi gel with MTGase increased from 2.40 to 3.30 mg/mL, and the solubility of proteins in the surimi gel without MTGase increased from 2.48 to 4.21 mg/mL. In general, with increase in the treatment pressure, the solubility of proteins was significantly increased; however, the degree of increase was lower in the surimi gel with MTGase. Balny and Masson[33] have reported that the high pressure processing treatment might break the covalent bonds in proteins, such as disulfide bonds, hydrogen bonds, ionic bonds, and hydrophobic bonds, resulting in an increase of soluble proteins. Thus, the surimi gel with MTGase contained well-preserved protein in spite of the high-pressure treatment. Simultaneous application of MTGase and high-pressure treatment can induce structural changes in proteins, causing the amino acids in the protein to become accessible to the acyl-binding site of MTGase[20]. The above results suggest that high-pressure treatment can effectively modify the conformation of proteins, and help MTGase in forming a better gelling network.

CONCLUSIONS

MTGase is widely used in the processing of food products. The simultaneous application of MTGase and high-pressure treatments provides exciting possibilities to improve the quality of tilapia surimi. The experimen-

tal results indicated that the activity of MTGase could be maintained up to 75% of its original level even after high-pressure treatment at 300 MPa for 120 min. The surimi gel containing MTGase (0.044 unit/g of paste), which was subjected to high-pressure processing, showed better gelation properties. Different dosages of MTGase and varying pressure treatments will be examined in forthcoming studies to obtain the optimal conditions for surimi processing.

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