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Transglutaminase effects on gelation capacity of thermally induced beef protein gels

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Abstract

Transglutaminase (TG) is an enzyme that catalyzes an acyl-transfer reaction between the γ-carboxyamide group of peptide or protein-bound glutaminyl residues, and primary amines. TG action on protein molecules, causes a cross-linking and polymerizing effect of these latter, through ε-(γ-glutamyl)lysine bonds. This TG-mediated protein cross-linking creates drastic physical changes in protein-rich foods.

This research objective, was to evaluate the microbial transglutaminase (MTGase) effect on beef gel texture. Time and incubation temperature, enzyme concentration, and Chilean jack mackerel (*Trachurus murphyi*) surimi addition effects, were studied.

Beef gel quality, was assessed by measuring gel strength and cooking yield. Additionally, SDS-PAGE electrophoresis and ε-(γ-glutamyl)lysine bond analysis by HPLC were carried out on the gels.

MTGase addition, significantly increased gel strength. The optimal attributes were observed at 60 °C after 2 h and 0.5% w/w of MTGase, with a gel strength 88% higher than the control-gel.

Jack mackerel surimi and MTGase incorporation in beef gels, significantly increased gel strength and cooking yield. SDS-PAGE analysis revealed that the myosin heavy chain (MHC) content decreased and that the cross-linked protein amount, apparently increased with time and MTGase addition. Proteolysis was also observed. ε-(γ-Glutamyl)lysine bond production also increased as a function of incubation time and MTGase concentration.

These results, suggest that it is possible to improve mechanical properties of beef gels by adding MTGase and jack mackerel surimi, thus increasing their potential utilization in minced based products.

Keywords: Transglutaminase; Gelation capacity; Beef; Surimi; Texture

1. Introduction

In the meat industry, high costs have stimulated research to utilize all animal parts to maximize the yield of marketable products. This includes the development of methods to restructure low-value cuts and trimmings to improve appearance and texture and so enhance market value. Restructuring usually involves size reduction, reforming and binding which is an essential requirement (Kim et al., 1993).

Transglutaminase (TG) is an enzyme that catalyzes an acyl-transfer reaction between the γ-carboxyamide group of peptide or protein-bound glutaminyl residues, and primary amines. TG action over protein molecules, causes a cross-linking and polymerizing effect of these latter, through ε-(γ-glutamyl) lysine bonds (Kuraishi et al., 1998). This TG-mediated protein cross-linking creates drastic physical changes in protein-rich foods.

Upon the discovery of an extracellular microbial TG, produced by a variant of *Streptoverticillium mobaraense,* utilization of MTGase in food industries was developed (Sakamoto et al., 1995). TG has been used to catalyse the cross-linking of a number of proteins, such as whey

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proteins, soya proteins, gluten, myosin and actomyosin (De Jong & Koppelman, 2002; Sakamoto, Kumasawa, & Motoki, 1994; Zhu, Rinzema, Tramper, & Bold, 1995).

It is considered that TG promotes the formation of crosslinks between myosin heavy chains (MHC) found in muscle proteins. Great improvements have been reported in the physical properties of the product, such as elasticity and firmness (Kuraishi, Yamasaki, & Susa, 2001).

Another ingredient which may be useful binding meat pieces is surimi, a water-washed, fish myofibrillar protein concentrate. Surimi has a unique ability, a setting phenomenon, which forms gels at relatively low temperatures (0–40 °C) (Torley & Lanier, 1991, chap. 32). Setting enhances the physical properties of firmness and cohesiveness in the fully cooked gels (Kim et al., 1993). Kumasawa, Seguro, Tamakura, and Motoki (1997), reported that, in this case, an endogenous transglutaminase (TG) is largely responsible for cross-linked polymers. There is no doubt that both endogenous fish TG and exogenous MTGase can improve the functionality of proteins by increased cross-linking. When an excess of TG is used, the texture becomes too firm and less pliable which is generally less acceptable. By controlling the amount of enzyme, reaction time and temperature, it is possible to obtain the desired texture (Seguro et al., 1995).

The objective of this study was to evaluate the microbial transglutaminase (MTGase) effects on beef gel texture. Incubation time and temperature, MTGase concentration and Chilean jack mackerel surimi (*Trachurus murphyi*)/beef proportion effects, were also studied.

2. Materials and methods

2.1. Materials

Boneless beef (top round) was purchased from Frigorífico Translink S.A. (Buenos Aires, Argentina). Surimi from Jack mackerel (*T. murphyi*) was supplied by Pesquera El Golfo (Talcahuano, Chile). Surimi and beef were stored at -18 ± 1 °C. Microbial transglutaminase from *Streptoverticillium mobaerense* (TG-K) was supplied by Ajinomoto Co. (Japan).

O-phthalaldehyde, synthetic ε-(γ-glutamyl) lysine, pronase, protease type XIV bacterial, carboxypeptidase A, leucine aminopeptidase and thymol were obtained from Sigma Chemical Co. All other chemical reagents were of analytical grade.

2.2. Preparation of gels

Partially thawed beef was mixed with 2.5% (w/w) NaCl and ice to adjust final moisture content to 76%. Each sample was blended under vacuum for 5 min, to a final temperature below 10 °C. The paste was then stuffed into stainless steel tubes (15×3 cm) using a hand-operated sausage stuffer. After the pre-incubation treatment, the samples were immediately cooked for 50 min at 90 °C. The resulting gels were immediately cooled in iced water for 10 min and stored overnight at 4 °C in plastic bags prior to gel testing (Dondero, Curotto, & Figueroa, 2002).

The following experiments were carried out:

- Incubation time and temperature: tubes were pre-incubated prior to a final cooking at 90 °C for 50 min. MTGase at 0.0% and 0.5% was added at each experiment: 4 °C: 0–6–12–24–36 h; 25 °C: 2–4–6–8 h; 45 °C: 2–4–6–8 h; 60 °C: 2–4–6–8 h.
- MTGase concentration effect: different levels of MTGase were incorporated into beef pastes (0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 1.0%, 2.0% and 3.0% w/w). The behaviour of gels heated directly at 90 °C for 50 min was also investigated.
- Paste composition: mixtures of beef/surimi (100:0, 75:25, 50:50, 25:75 and 0:100) were studied. Tubes were incubated at 60 and 25 °C for 2 h, prior to cooking. Each experiment was performed with (0.5%) and without TG.

2.3. Gel texture analysis

Breaking strength (g) and deformation (cm) were measured in a TATX2 Texturometer (Texture Technology Corporation, USA) by a Texture Expert Software vs. 1.0. The instrumental conditions were: speed 0.5 mm; distance 23 mm; probe diameter 5 mm. The analysis was done on 18 samples treated by the same procedure. Average and standard deviations were calculated. Gel strength was calculated as the product of breaking strength by deformation ($g \times cm$).

2.4. SDS-polyacrylamide gel electrophoresis

SDS-PAGE was performed according to the procedure proposed by Laemmli (1970). Ten percentage and 7% (w/v) polyacrylamide were used as separating gels. Gels were solubilized according to the method of Nishimoto et al. (1988).

2.5. Analysis of ε-(γ-glutamyl) lysine dipeptide

Analysis of ε-(γ-glutamyl)lysine dipeptide in the gel was performed as described by Kumasawa et al. (1997). Briefly, gel samples were subjected to an enzymatic digestion with pronase, leucine aminopeptidase, prolidase and carboxypeptidase A, as described by Kumazawa, Numasawa, Seguro, and Motoki (1995). The digested samples were then lyophilized. Derivatization with O-phtalaldehyde (OPA) and fractionation by high performance liquid chromatography (HPLC) were performed according to the procedure of Griffin, Wilson, and Lorand (1982). Elution time and ε-(γ-glutamyl)lysine content were estimated by using synthetic ε-(γ-glutamyl)lysine as a reference. The experiment was done with an intelligent pump Merck–Hitachi

model L-6200A; a fluorescent detector (Perkin–Elmer model LS-30) and a chromatographic integrator (Merck–Hitachi model D-2500).

2.6. Cooking yield determination

The yield of the meat gels was calculated as the weight of the cooked meat-gel divided by the weight of the non-cooked meat paste, expressed as a percentage (Tseng, Liu, & Chen, 2000).

2.7. Statistical analysis

Statistical analysis of data was carried out using the Duncan test at $p \leqslant 0.05$. This analysis was performed using Statgraphics vs. 5.0 and Excel 97 computational programmes.

3. Results and discussion

3.1. Effects of incubation time and temperature

Gels with 0.5% MTGase always presented significantly ($p \leqslant 0.05$) higher gel strength values than did gels without MTGase (Fig. 1). Gels incubated with MTGase at 60 °C always presented higher values than those incubated at 4, 25 or 45 °C. Gel strength with 0.5% MTGase, at 60 °C over 2 h was 88% higher than the control-gel. With longer incubation time, the gel strength decreased as was also observed by Sakamoto et al. (1995) with surimi from Alaska pollack and MTGase. They hypothesized that excessive formation of ε-(γ-glutamyl)lysine cross-link would inhibit uniform development of the protein networks affecting breaking strength, deformation or gel strength.

The treatments with MTGase were significantly higher with respect to control for all temperatures and times of incubation ($p \leqslant 0.05$). Samejima, Egelandsdal, and Fretheim (1985), has reported that beef myofibrils were found to start forming gels at 43–56 °C, however, maximum gel strength in jack mackerel surimi (*T. murphyi*) has been reported to occur at 25 °C (Dondero et al., 2002). Several studies, have indicated that the thermal stability of muscle proteins increases with higher normal body temperatures of animals (Rodgers, Karr, Biedermann, Ueno, & Harrigton, 1987).

Mammalian species, such as beef, pork and whale, are classified as non-setting species (Niwa, Suzumura, Nowsad, & Katoh, 1993). Absence of a setting response in beef meat may be due to either a lack of cross-linking enzyme activity and/or a lower availability of substrate for enzyme activity at low temperatures. If transglutaminase activity is lacking, this may be due to absence of the enzyme or to a lack of cofactors (Kim et al., 1993). However, optimum temperature for endogenous beef TG was found to be 40 °C by Muñoz (1999).

Gels incubated at low temperatures, always presented significantly ($p \leq 0.05$) higher cooking yield values, than did gels incubated at higher temperatures (Fig. 2). The treatments with MTGase were not significant with respect

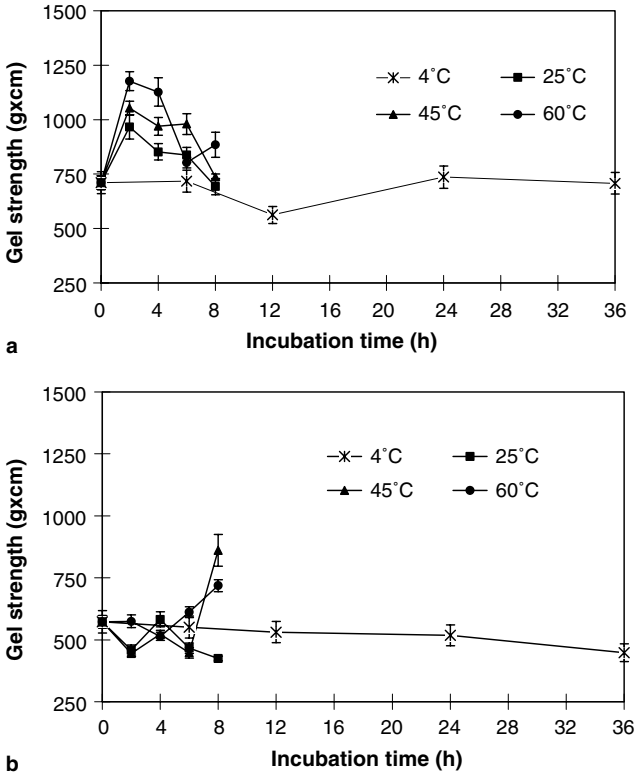


Fig. 1. Effects of incubation time and temperature on gel strength of beef gels: (a) with 0.5%MTGase and (b) without MTGase.

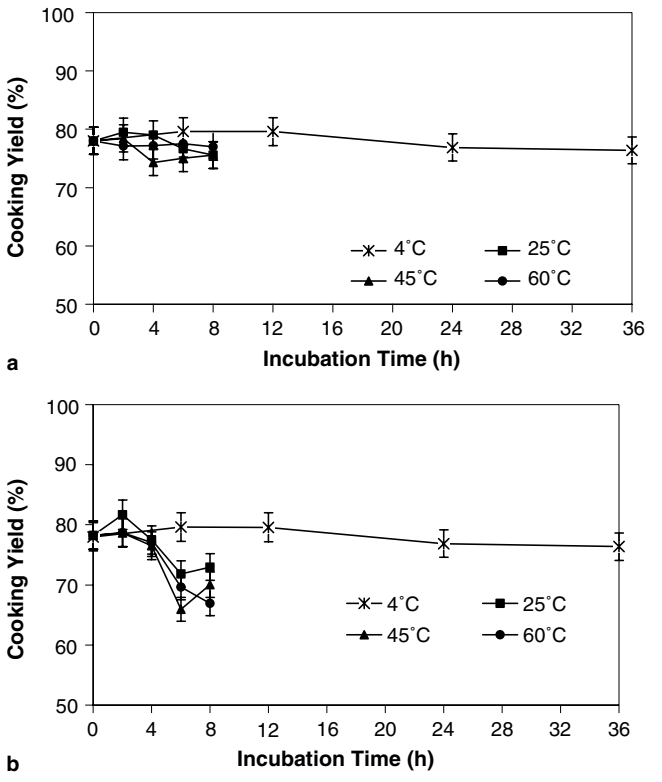


Fig. 2. Effects of incubation time and temperature on cooking yield of beef gels: (a) without MTGase and (b) with 0.5% MTGase.

to the treatment without MTGase ($p \leqslant 0.05$). In general, the cooking loss of meat products on heat treatment decreases with increasing salt concentration (Samejima et al., 1985). Pietrasik and Li-Chan (2002) observed that MTGase addition favourably reduced the cooking loss of pork batter gels. However, this effect has not been reported in all cases (Numata, Yamada, Nakamura, & Muguruma, 1989).

Through the incubation time at 60 °C, without MTGase, the myosin band still persisted after 8 h incubation time and degradation of MHC was also observed. In samples without MTGase, the degradation products due to proteolysis were higher than in samples with MTGase (Fig. 3). With prolonged incubation time with MTGase, a decrease in the MHC band was observed. These results suggest that cross-linking and degradation of the MHC occurred simultaneously during incubation at 60 °C. However, no obvious change in actin band was observed through the incubation time. This was also observed by De Backer-Royer, Traorë, and Meunier (1992) and Tsai, Lin, and Jiang (1996). Yanusaga, Abe, Yamasawa, and Arai (1996), incubated gels from Walleye Pollack (*Theragra chalcogramma*) at 25 °C with 0.3% TG, and found that on increasing the incubation time, a sharp decrease in myosin content and an increase in polymer content were observed. Kim et al. (1993), observed polymerization of beef actomyosin by TG. After 30 min of incubation at 35 °C, early stages of network formation and aggregation were apparent. After 60 min, extensive aggregation was readily visualized. Cross-linking of proteins results in formation of dimers, trimers and larger protein polymers (De Jong & Koppelman, 2002).

Gels incubated at 60 °C, with 0.5% MTGase always showed a significantly ($p \leqslant 0.05$) higher content of ε-(γ-glutamyl) lysine dipeptide than did those without MTGase (Fig. 4). Through the time at 60 °C, the ε-(γ-glutamyl)lysine dipeptide increased, reaching 41.3 μmol/g protein after 8 h of incubation. Levels of 32.3 μmol/g protein were detected in gels from jack mackerel surimi after 8 h of setting at 25 °C with 0.2%w/w MTGase (Dondero et al., 2002).

The efficiency at which a protein can be utilized as a substrate by TG, seems to be influenced by the amino acid sequence around the reactive glutamines. Thus, the primary structure of a protein is of greater importance in assessing its ability to act as a substrate than its absolute lysine and glutamine contents (Kurth & Rogers, 1984). Beef myosin is apparently able to form tetramers when treated with beef blood TG, so it apparently has lysine and glutamine sites available (Kurth & Rogers, 1984). But, excessive formation of ε-(γ-glutamyl)lysine dipeptide would inhibit uniform development of the heat-induced protein network and the improvement of breaking strength or deformation or gel strength (Sakamoto et al., 1995). This was also observed in Fig. 1 with beef gels. It describes a relationship between ε-(γ-glutamyl)lysine dipeptide and gel strength, until the content of ε-(γ-glutamyl)lysine bond was below 3 μmol/100 g in gels from Alaska Pollack (Seguro et al., 1995). This behaviour might occur because an increment of protein-protein interactions decreases the water–protein interactions and consequently result in a decrease in deformation.

3.2. Effects of enzyme concentration

The changes in textural characteristics varied with MTGase concentrations. Gels incubated at 60 °C prior to cooking, always showed significantly ($p \leqslant 0.05$) higher gel

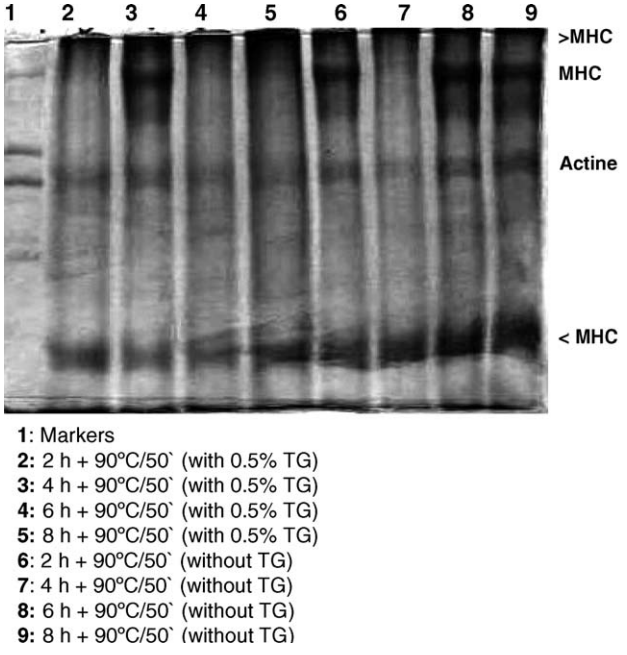


Fig. 3. SDS-PAGE pattern of beef gels as related to incubation time at 60 °C, with 0.5% and without MTGase.

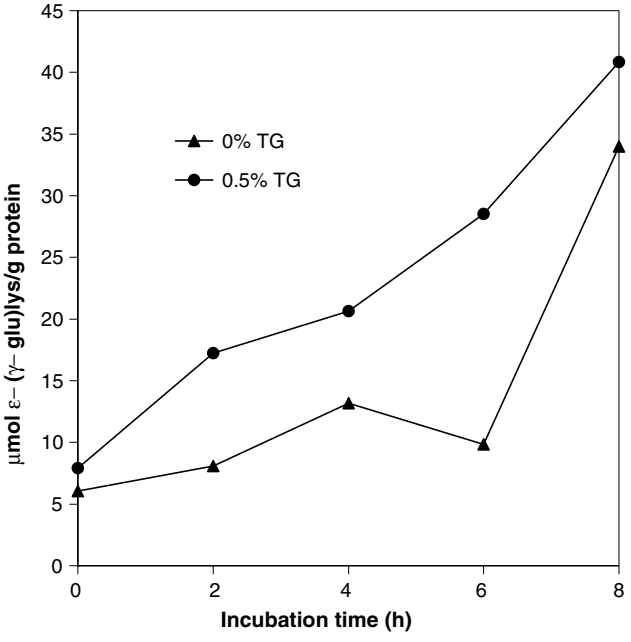


Fig. 4. Changes in ε-(γ-glutamyl)lysine content in beef gels as related to incubation time at 60 °C.

strength than did gels without incubation (Fig. 5). Gel strength in gels incubated for 2 h at 60 °C, increased significantly ($p \leqslant 0.05$) to 0.5% MTGase level, reaching an increase of 88% in comparison to control without TG. A pre-requisite for the cross-linking reaction with TG is a sufficient exposure of the lysines and glutamines of substrate protein (De Jong & Koppelman, 2002).

During direct cooking, MTGase catalyzed the reaction until it became totally inactivated. MTGase lost activity, within a few minutes, on heating at 70 °C (Motoki & Seguro, 1998).

Dondero et al. (2002) reported that the maximum gel strength in surimi from jack mackerel (*T. murphyi*) was observed with 0.2%TG at 25 °C over 2 h with an increase of 364% over control without TG. Polymerization, induced by TG, depends on species, and might be due to substrate specificity (Asagami, Ogiwara, Wakameda, & Noguchi, 1995). Food proteins with rigid structures prevent cross-linking (De Jong & Koppelman, 2002).

Cooking yield decreased significantly ($p \leqslant 0.05$) as MTGase level increased. This was probably due to stronger interactions between protein molecules excluding water (Fig. 6). Tseng et al. (2000) observed that the yield of low-salt chicken meat-balls increased with higher percentage MTgase, suggesting that MTgase improves emulsion stability and hydration properties.

The SDS-PAGE pattern showed a decrease in the myosin heavy chain band (MHC) as MTGase concentration increased (Fig. 7). Therefore, polymers were formed by covalent cross-links catalyzed by MTGase. Actin did not participate in the cross-linking as its intensity was unchanged at all studied MTGase concentrations. Sakamoto et al. (1995) also found a decrease in myosin bands in gels with and without setting (30 °C over 1 h), when higher levels of TG were used in Alaska pollack surimi (*T. chalcogramma*). Nishimoto, Hashimoto, Seki, and Arai (1987) reported that MHC from Alaska pollack decreased and polymer content increased with 0.2% TG. They suggested that the decrease in myosin band would be evidence of the formation of covalent cross-links.

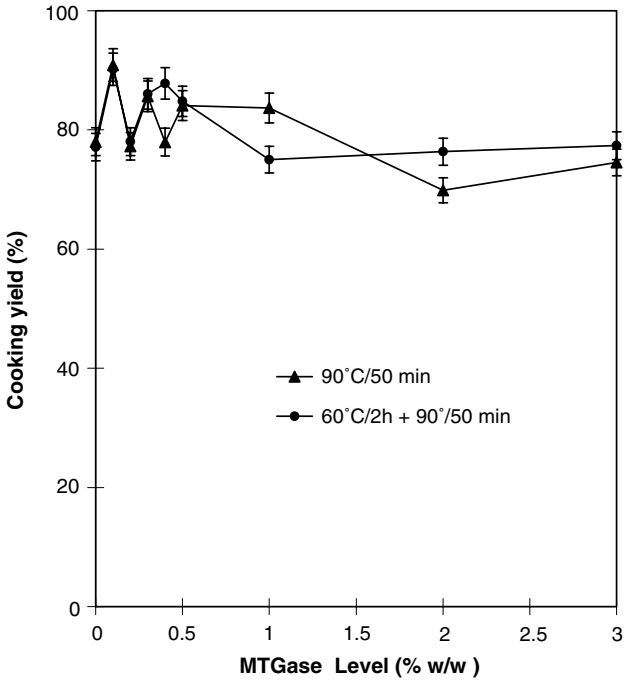


Fig. 6. Changes in cooking yield of beef gels as related to MTGase level.

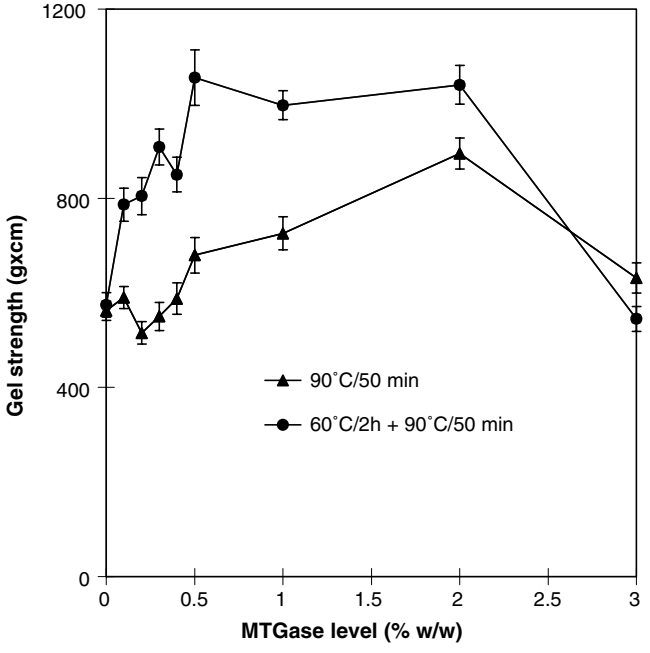
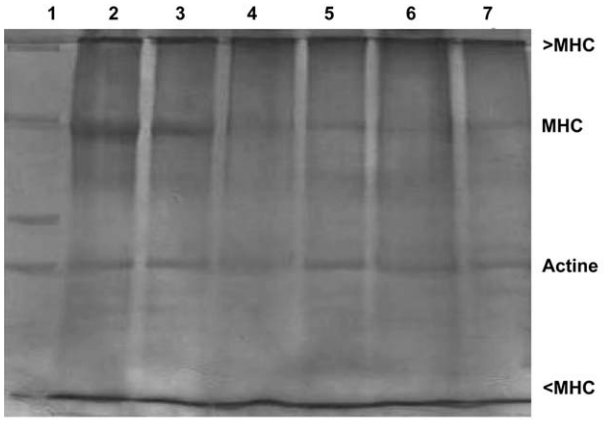


Fig. 5. Changes in gel strength of beef gels as a function of MTGase concentration.



1: Markers
2: 0.05% TG
3: 0.1% TG
4: 0.2% TG
5: 0.3% TG
6: 0.4% TG
7: 0.5% TG

Fig. 7. SDS-PAGE pattern of beef gels incubated at 60 °C, as related to transglutaminase level.

ε-(γ-Glutamyl)lysine bonds catalyzed by TG are shown in Fig. 8. Gels incubated for 2 h at 60 °C, showed higher amounts of ε-(γ-glutamyl) lysine dipeptide when MTGase concentration increased. Sakamoto et al. (1994) observed in soy protein isolate and egg white gels, that the amount of ε-(γ-glutamyl)lysine bonds increased with MTGase concentration. The monomeric fraction of the intact proteins diminished and a novel polymer fraction, which could not enter the gradient gel, was formed. Also, during direct cooking, ε-(γ-glutamyl) lysine dipeptide increased with an increase of MTGase level. Sakamoto et al. (1995), suggested that high temperatures (121 °C) can produce protein condensation reactions, also forming ε-(γ-glutamyl) lysine dipeptide.

3.3. Effects of mixture composition: jack mackerel surimi/beef

Gels were incubated at 25 and 60 °C over 2 h, optimum temperatures for surimi and beef gelation, respectively (Curotto, Canales, Alviña, & Dondero, 1999; Dondero et al., 2002). Without TG, gel strength decreased significantly ($p \leqslant 0.05$) with decreasing beef content whereas, with TG, gel strength increased significantly ($p \leqslant 0.05$) when surimi content increased (Fig. 9).

The weakening of the mixed protein gels may be ascribed to the interruption of the surimi matrix by the beef gel or viceversa (Kim et al., 1993). Torley and Lanier (1991, chap. 32) proposed that, in beef/Alaska pollock surimi (*T. chalcogramma*) mixtures, the Alaska pollock muscle transglutaminase was unable to catalyze isopeptide cross-link formation in beef myosin, depending on whether the myosin of beef was in an non-reactive form, or some fraction of the beef muscle may inhibit the cross-linking enzyme, or a protein with only a single site capable of forming an isopeptide cross-link may bind to the polymer and stop its growth. The decrease in strength of the surimi gel may also be due, in part, to the decrease in pH from that which is optimal for surimi with beef addition (Kim et al., 1993). In this study, the 100% surimi had a pH of 6.85, decreasing to pH 5.95 in 100% beef. Jack mackerel surimi reportedly has highest gel strength at pH 7.0 (Figueroa, 1997) and beef gels at pH 6.0 (Trout and Schmidt, 1986). Incorporation of beef myofibrils into Alaska pollock surimi resulted in a decrease in shear stress and true shear strain values (Kim et al., 1993).

At 60 °C, without MTGase, gel strength decreased dramatically when surimi content increased, due to the softening of gels (modori) closely related to the breakdown of myosin heavy chain caused by a group of alkaline heat stable proteases found in surimi from jack mackerel (Curotto et al., 1999; Dondero, Concha, & Curotto, 1999). The presence of MTGase arrested this effect, and the gel strength was always higher with MTGase than without MTGase. The mixture 75% surimi/25% beef, showed the highest gel strength, increasing 388% and 299%, at 60 and 25 °C, respectively, compared with their control without TG and incubation. MTGase addition at 60 °C, gave better results in textural properties with all mixtures.

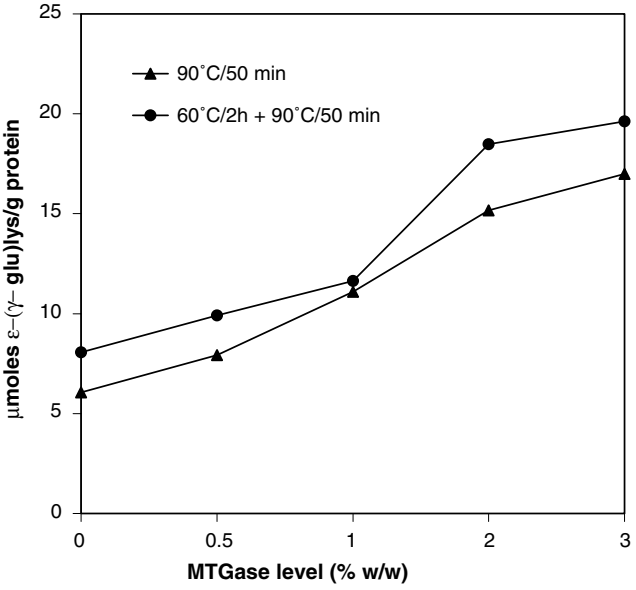


Fig. 8. Changes in ε-(γ-glutamyl)lysine content in beef gels as related to MTGase concentration.

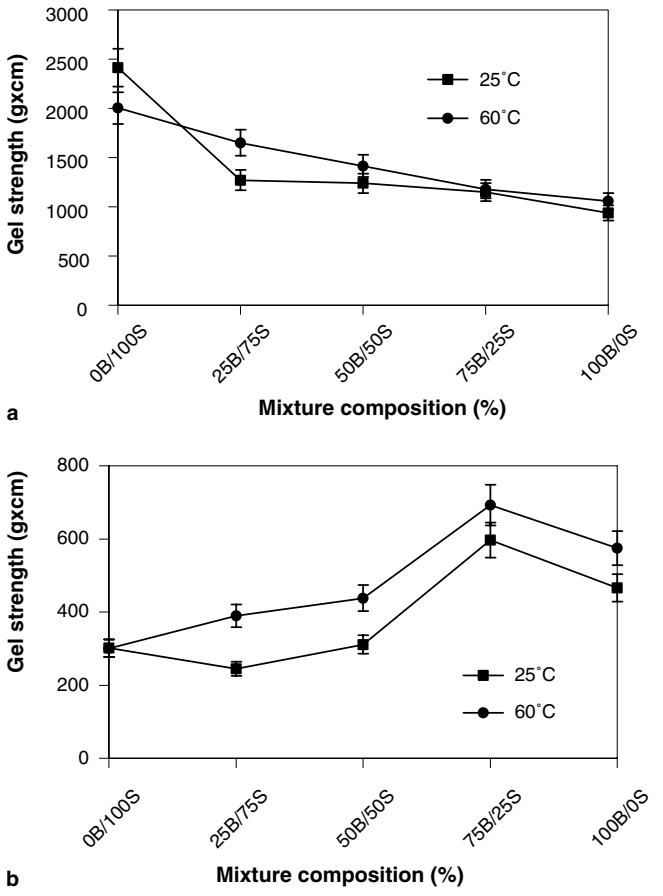


Fig. 9. Effects of mixture composition beef(B)/jack mackerel surimi(S) on gel strength: (a) 0.5% MTGase and (b) 0% MTGase.

With MTGase, cooking yield decreased significantly ($p \leqslant 0.05$)when beef content increased at 25 and 60 °C ($p \leqslant 0.05$). At 60 °C, the cooking yield was lower than at 25 °C (Fig. 10). Beef samples always presented higher fat contents than did jack mackerel surimi. Fat will melt at a higher temperature.

At 25 °C, with MTGase, the SDS-PAGE pattern showed that the myosin band decreased, compared to gels without MTGase, where the myosin band still persisted. The amounts of MHC apparently decreased with increased surimi concentration. In the 100% surimi sample with MTGase, the myosin band almost disappeared. Polymers were also observed. However, the actin band was unchanged with changing mixture composition (Fig. 11).

At 60 °C, with MTGase, the myosin band decreased in all samples, mainly with 100% surimi (Fig. 12). Without TG, the myosin band still persisted. The lower intensity of the MHC band in the 100% surimi sample might suggest a higher activity of its endogenous TG compared with endogenous beef TG. Furthermore, myosin degradation was greatly accelerated at 60 °C, where proteolysis of jack mackerel surimi reached an optimum (Dondero et al., 1999). Degradation products were observed due to proteolysis which was more evident without MTGase.

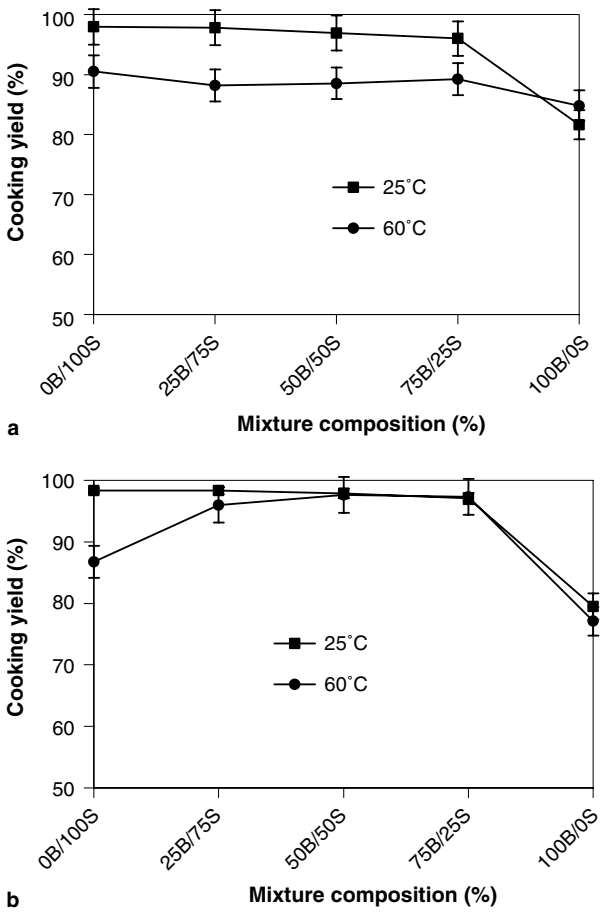
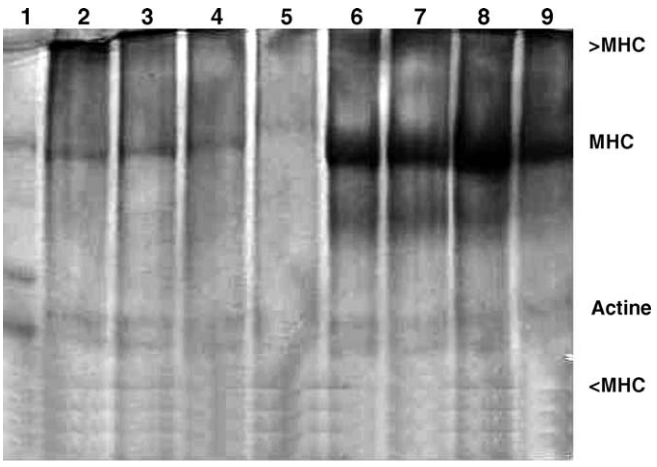
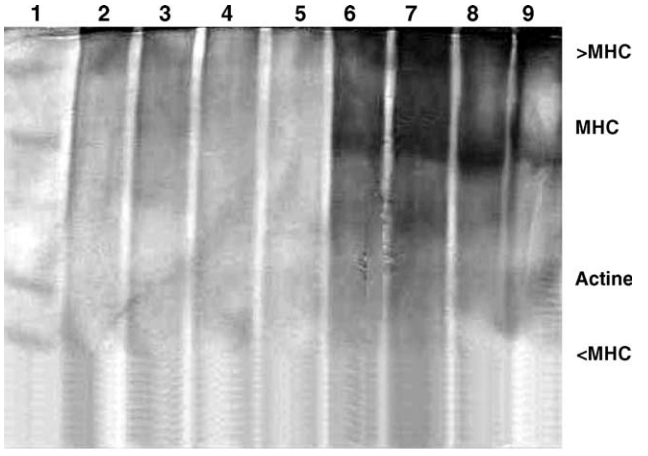


Fig. 10. Effects of mixture composition of beef(B)/jack mackerel surimi(S) on cooking yield: (a) with 0.5% MTGase and (b) without MTGase.



1: Markers
2: 25% surimi–75% beef 0.5% TG
3: 50% surimi– 50% beef 0.5% TG
4: 75% surimi– 25% beef 0.5% TG
5: 100% surimi 0.5% TG
6: 25% surimi–75% beef 0% TG
7: 50% surimi–50% beef 0% TG
8: 75% surimi–25% beef 0% TG
9: 100% surimi 0% TG

Fig. 11. SDS-PAGE pattern as related to mixture composition of beef/jack mackerel surimi at 25 °C.



1: Markers
2: 25% surimi–75% beef 0.5% TG
3: 50% surimi– 50% beef 0.5% TG
4: 75% surimi– 25% beef 0.5% TG
5: 100% surimi-- 0.5% TG
6: 25% surimi–75% beef 0% TG
7: 50% surimi–50% beef 0% TG
8: 75% surimi–25% beef 0% TG
9: 100% surimi-- 0% TG

Fig. 12. SDS-PAGE pattern, as related to mixture composition of beef/jack mackerel surimi at 60 °C.

Gels incubated at 25 °C, showed higher contents of ε-(γ-glutamyl)lysine dipeptide in mixtures than did gels at 60 °C (Fig. 13). This temperature might block the MTGase performance. In addition, jack mackerel surimi has endogenous TG activity, which improves at 25 °C whereas beef TG presents an optimum temperature at 40 °C (Muñoz, 1999). Probably,

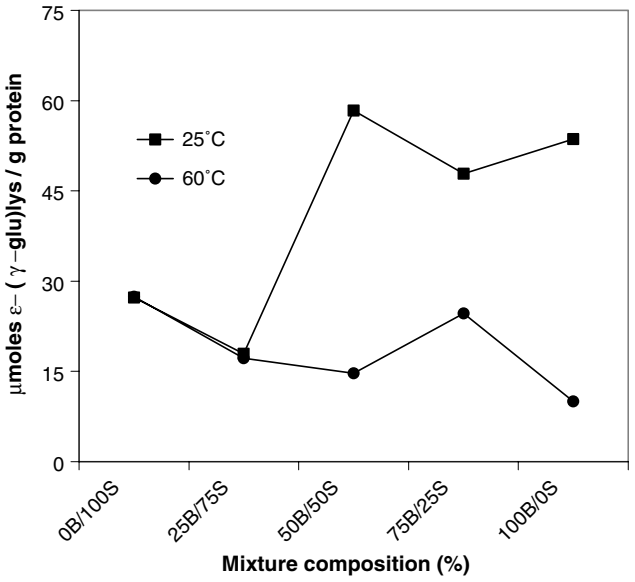


Fig. 13. Changes in ε-(γ-glutamyl)lysine content, as a function of mixture composition of beef(B)/jack mackerel surimi(S) with 0.5% MTGase.

a synergisic effect between endogenous and exogenous TG still exists at 25 °C. The highest value for ε-(γ-glutamyl)lysine bonds was found in the 50% beef/50% surimi mixture. A correlation between gel strength and ε-(γ-glutamyl)lysine dipeptide was not observed, probably due to the decrease in deformation of the gels (data not shown).

Ramírez-Suarez and Xiong (2003), concluded that whey proteins do not cross-link with muscle proteins through ε-(γ-glutamyl)lysine isopeptide bonds, but they appear to promote the enzyme-catalyzed intra- and inter-molecular association and reinforce the myofibrillar protein gel network through another mechanism when heated above 75 °C. The improvement of gel strength can be attributed to non-covalent forces (such as hydrophobic and electrostatic interactions) and possibly also to disulfide cross-links. Later, these authors (2003) reported that MTGase induced cross-linking in gelation of a myofibrillar/soy protein mixtures producing an adhesive mixed protein gel structure with a reduced concentration requirement for extracted myofibrillar proteins. These interactions led to ordered heteropolymers or aggregates that produced gels with greater storage moduli. In general, the TG will prefer one of the two proteins. Consequently, polymers of this protein will be formed first (Han & Damodaran, 1996). The other protein will be cross-linked at a later stage, either to itself or to the high molecular weight polymer of the first protein. Thus, it is very difficult to ensure that two different proteins are actually coupled and that not only large polymers of the separate proteins are formed (De Jong & Koppelman, 2002).

4. Conclusions

MTGase addition and incubation significantly improved ($p \leqslant 0.05$) the textural properties in beef gels. The optimal attributes were observed at 60 °C for 2 h and 0,5% w/w of TG, with a gel strength 88% higher than the control-gel.

The cooking yield decreased significantly ($p \leqslant 0.05$) with the addition of TG, higher temperatures and longer incubation time.

Jack mackerel surimi and MTGase incorporation into beef gels, significantly increased the cooking yield and gel strength. The 75% surimi/25% beef mixture, showed the highest gel strength and increases of 388% and 299%, at 60 and 25 °C, respectively, compared with their control without MTGase and incubation.

Higher content of ε-(γ-glutamyl) lysine dipeptide were found when incubation time and MTGase concentration increased.

SDS-PAGE patterns showed that the myosin heavy chain (MHC) band decreased, and that the cross-linked protein apparently increased with increasing time and MTGase addition. Proteolysis was also observed.

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