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# Effects of combining microbial transglutaminase and high pressure processing treatments on the mechanical properties of heat-induced gels prepared from arrowtooth flounder (*Atheresthes stomias*)

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**Abstract**

The objective of this study was to evaluate the effect of setting conditions (25 °C for 2 h or 40 °C for 30 min) and combining of microbial transglutaminase (MTGase) and high pressure processing (HPP) on the mechanical properties of heat induced gels obtained from paste from arrowtooth flounder (*Atheresthes stomias*). Treatments included fish paste control without added MTGase, fish paste incubated with MTGase but not pressurized (MTGase + cooking), fish paste incubated with MTGase and pressurized at 600 MPa for 5 min (MTGase + HPP + cooking) and fish paste pressurized at 600 MPa for 5 min and incubated with MTGase (HPP + MTGase + cooking). The controls and the treated samples were then subjected to one of two thermal treatments: 90 °C for 15 min or 60 °C for 30 min before cooking at 90 °C for 15 min. Samples of fish paste heated at 60 °C before cooking could not be used to prepare gels for texture profile analysis (TPA). TPA showed that pressurization improved the mechanical properties of gels made from paste treated with MTGase and set at 25 °C. The opposite was observed for samples set at 40 °C. Setting at 40 °C appeared to induce proteolytic degradation of myofibrillar proteins.



*Keywords:* High pressure; *Arrowtooth flounder*; *Atheresthes stomias*; Setting; TPA; Proteolysis

## 1. Introduction

Arrowtooth flounder (*Atheresthes stomias*) is the most abundant fishery resource in the Gulf of Alaska and even greater than Alaskan Pollock (*Theragra chalcogramma*) (Regenstein, 2004). However, it has low commercial value because its flesh texture degrades during heating. Efforts to improve the commercial value of this species have been reported recently (Uresti, Velázquez, Ramírez, Vázquez, & Torres, 2004a, 2004b). Many fish species show protein degradation during heating, a phenomenon extensively studied in kamaboko manufacture, and called ‘‘modori’’. Myosin, the myofibrillar protein responsible for functional and

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mechanical properties, is the main substrate for heat stable proteolytic enzymes responsible for this quality loss (An, Margo, & Seymour, 1996; Ramírez, García-Carreño, Morales, & Sánchez, 2002). Maximum myofibrillar protein degradation occurs at 50–70 °C. In arrowtooth flounder, a heat-stable cysteine protease with similar properties to cathepsin L and molecular weight of 33 kDa, has been associated with proteolysis during cooking (Izquierdo Pulido et al., 1996).

The mechanical properties of surimi can be enhanced by incubation at temperatures below 40 °C. This phenomenon, reflecting protein crosslinking induced by an endogenous calcium-dependent transglutaminase (TGase), is called setting or suwari. TGase catalyzes a covalent bond between the ε-amino group of lysyl residues and the γ-carboxyamide group of glutaminyl residues of adjacent proteins (Kumazawa, Seguro, Takamura, & Motoki, 1993; Seki, Nakahara, Takeda, Maruyama, & Nozawa, 1998). The optimal conditions for this enzyme depend on the fish habitat temperature. Cold water fish, such as Alaska Pollock (*T. chalcogramma*) and Pacific whiting (*Merluccius productus*), show greater mechanical properties when set at temperatures below 25 °C while fish from warm water set better when incubated at 40 °C. The temperature effect on setting seems to be more associated with the myosin denaturation/aggregation temperature than with the temperature for optimal activity of the endogenous TGase (Lee & Park, 1998; Ramírez, Rodríguez-Sosa, Morales, & Vázquez, 2000, 2003; Uresti, Téllez-Luis, Ramírez, & Vázquez, 2004). A non-calcium dependent microbial transglutaminase (MTGase) is commonly used by industry to improve the mechanical properties of meat and fish products.

Interest in safer fish products by high pressure processing (HPP) is increasing. This technology can be used to obtain surimi gels and restructured fish products (Jiménez-Colmenero, 2002). HPP treatments at non-denaturizing temperatures (below 10 °C) have been shown to improve the mechanical properties of fish gels (Borderías, Pérez-Mateos, & Solas, 1997; Fernández-Martín, Pérez-Mateos, & Montero, 1998; Uresti, Ló-

Table 1
Significance level obtained, from analysis of variance, for the effect of setting temperature and MTGase/HPP treatments on the TPA parameters of arrowtooth flounder gels

| Source of variation | Hardness | Fracturability | Springiness | Cohesiveness | Chewiness |
|---|---|---|---|---|---|
| Main effects | | | | | |
| Setting temperature | 0.0000 | 0.0000 | 0.0245 | 0.0521 | 0.0000 |
| MTGase/HPP treatments | 0.0012 | 0.0000 | 0.0669 | 0.0000 | 0.0012 |

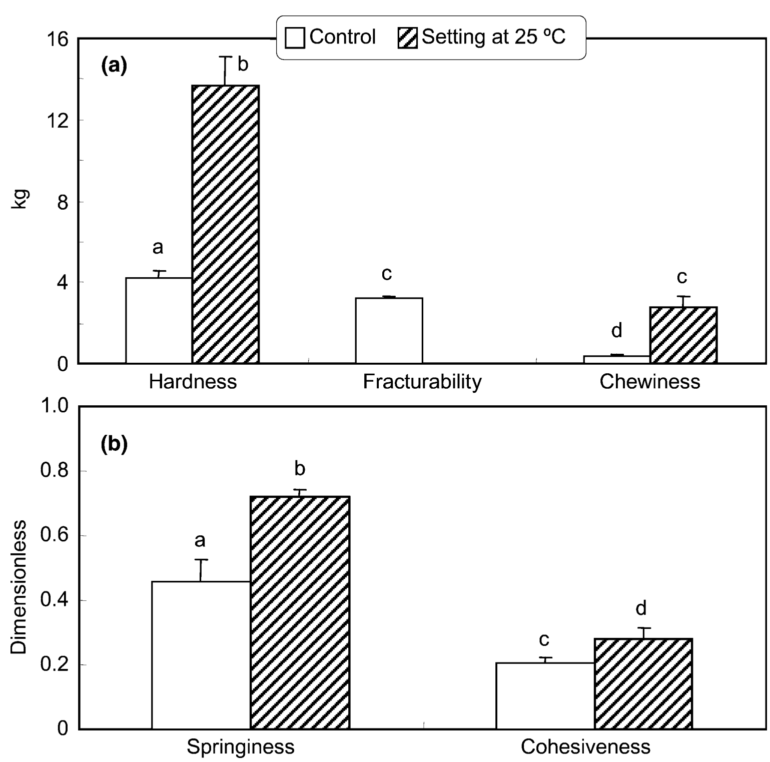


Fig. 1. Effect of MTGase on the TPA parameters of raw gels from fish paste from arrowtooth flounder (*Atheresthes stomias*) mince. Different letters indicate differences ($p < 0.05$) between treatments (columns).

pez-Arias, González-Cabriales, Ramírez, & Vázquez, 2003). The HPP effects on the mechanical and functional properties of raw and cooked fish gels obtained from salt-solubilized arrowtooth flounder paste have been previously reported (Uresti et al., 2004a, Uresti, Velázquez, Vázquez, Ramírez, & Torres, 2004b,Uresti et al., 2004c). These studies showed that fish gels with improved mechanical properties can be obtained by treating arrowtooth flounder paste at 400–600 MPa for 5 min. A different mechanism of protein aggregation was proposed for pressure-induced as compared to heat-induced fish gels. It seems that HPP treatment of fish paste at low temperature induces an aggregation characterized by side-to-side interactions of proteins with a low degree of denaturation and not by aggregation of proteins with large changes in molecular conformation. Because of this, pressure-induced aggregation improved the mechanical properties of heat-induced gels, a similar situation to the favourable protein aggregation induced at low temperature by TGase. However, HPP did not inhibit the proteolytic activity during cooking (Uresti et al., 2004a, 2004b, Uresti, Velázquez, Vázquez, Ramírez, & Torres, 2004c). The objective of this work was to determine the effects of setting conditions (25 °C for 2 h or 40 °C for 30 min), the addition of MTGase and HPP on the mechanical properties of heat induced fish gels obtained from fish paste produced from arrowtooth flounder mince.

## 2. Materials and methods

### 2.1. Fish mince

Fresh fillets of arrowtooth flounder (*Atheresthes stomias*) purchased from a local fish market (Corvallis, OR) were transported on ice to the laboratory and thoroughly rinsed with cold tap water. Fish mince was prepared using a Hobart food processor (Model A-200, Troy, OH) with a 5 mm diameter mesh and blended at <10 °C for 5 min with 20 g $kg^{-1}$ of salt using its mixer attachment. The resulting paste was stuffed into commercial 20 mm diameter sausage cases and stored at 4 °C until analyzed (~12 h). The same procedure was used to prepare samples containing 3 g $kg^{-1}$ of MTGase which was added to the fish mince during the mixing with salt. All samples were placed in a polystyrene bag for the HPP and thermal treatments described below.

### 2.2. High pressure processing treatments

Fish paste was treated at 600 MPa for 5 min using a 22 l high pressure vessel (Engineered Pressure Systems Inc., Haverhill, MA) filled with crushed ice and water. This vessel is equipped with a 40 HP pressure intensifier (Avure Technologies, Inc., Kent, WA), allowing a come up time to 600 MPa of ~40 s.

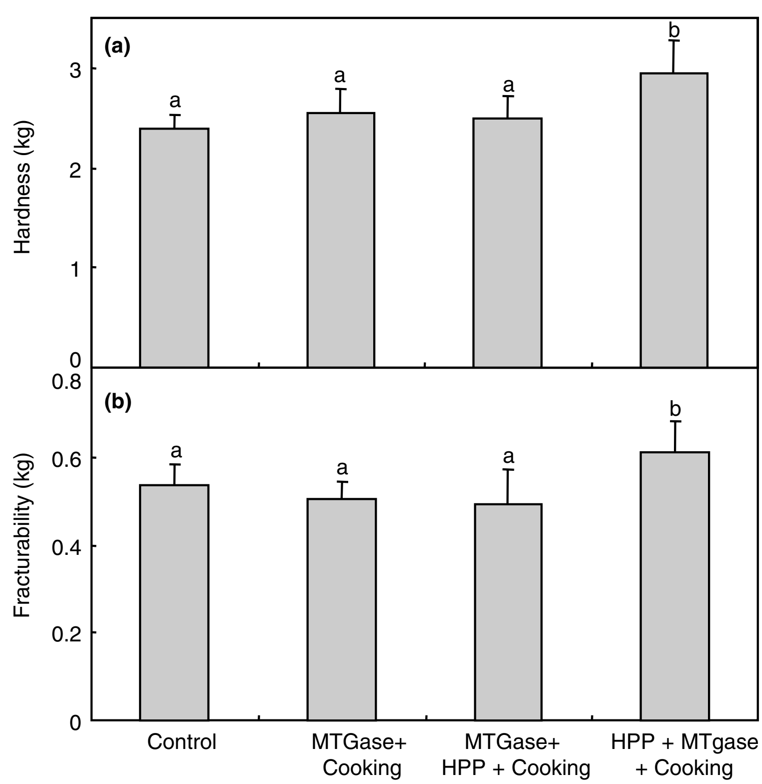


Fig. 2. Effect of MTGase and HPP on the hardness and fracturability of heat-induced gels from from fish paste from arrowtooth flounder (*Atheresthes stomias*) mince incubated at 25 °C for 2 h. Different letters indicate differences ($p < 0.05$) between treatments (columns).

### 2.3. Thermal treatments

The effects of added MTGase and HPP treatment on the mechanical properties of restructured fish products were studied by setting fish pastes from arrowtooth flounder at 25 °C for 2 h or at 40 °C for 30 min. Treatments included: (1) fish paste without MTGase (control); (2) fish paste with MTGase incubated at both setting conditions but not pressurized (MTGase + cooking); (3) fish paste with MTGase incubated at both setting conditions and then pressurized at 600 MPa for 5 min (MTGase + HPP + cooking); and, (4) fish paste pressurized at 600 MPa for 5 min before incubating at both setting conditions with MTGase (HPP + MTGase + cooking). The controls and the treated samples were then subjected to one of two thermal treatments: 90 °C for 15 min or 60 °C for 30 min before cooking at 90 °C for 15 min. Immediately after the thermal treatments, all samples were immersed for 30 min in a water bath at 4–5 °C.

### 2.4. Textural profile analysis

Cylindrical samples of the heat-set gels of 20 ± 1 mm diameter and 20 ± 2 mm length were placed in a plastic bag to avoid dehydration during equilibration to room temperature for 30 min. Textural profile analysis (TPA) was performed using a TA-XT2 texturometer (Stable Micro Systems, Vienna Court, England). Samples were compressed to 75% of initial height using a compression speed of 60 mm $min^{-1}$ and an aluminium cylindrical probe (P/50) of 50 mm diameter. Hardness, fracturability, springiness, cohesiveness and chewiness, defined as previously reported (Uresti et al., 2004a),

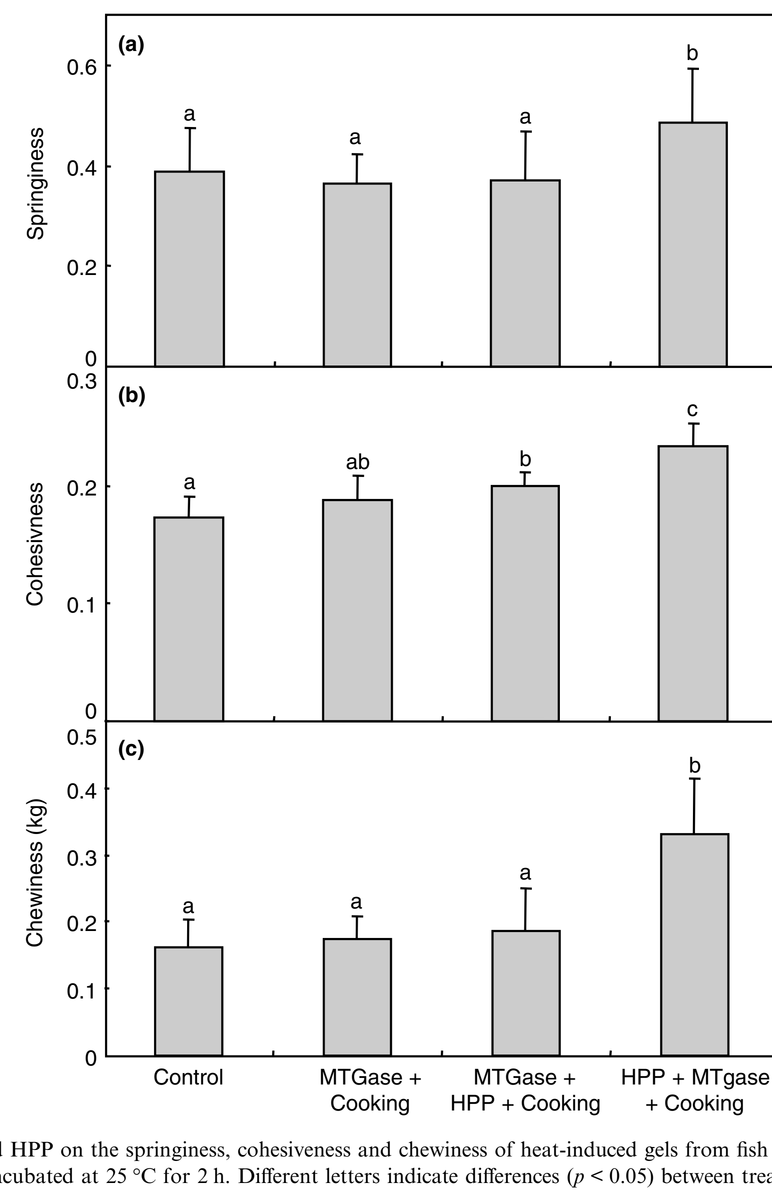


Fig. 3. Effect of MTGase and HPP on the springiness, cohesiveness and chewiness of heat-induced gels from fish paste from arrowtooth flounder (*Atheresthes stomias*) mince incubated at 25 °C for 2 h. Different letters indicate differences ($p < 0.05$) between treatments (columns).

were determined for each treatment. Ten samples were analyzed for each treatment.

### 2.5. Statistical analysis

Data were analyzed by multifactorial analysis of variance (Statgraphics Ver. 5, Manugistics, Inc. Rockville, MD). Differences among mean values were established using the least significant difference (LSD) multiple range test and were considered significant when $p < 0.05$.

## 3. Results and discussion

### 3.1. General

The samples of fish paste incubated at 60 °C (temperature of maximum proteolytic activity) for 30 min before cooking at 90 °C for 15 min were totally liquefied and could not be used to prepare gels. This showed that MTGase, HPP and their combinations were unable to inhibit the heat-stable proteolytic enzymes responsible for myofibrillar protein degradation. The results of analysis of variance on the effect of setting temperature (25 or 40 °C) and MTGase/HPP treatments for samples cooked at 90 °C for 15 min are shown in Table 1. Both factors, setting temperature and MTGase/HPP treatments, significantly affected most TPA parameters. The exceptions were cohesiveness which was not affected by setting temperature ($p = 0.0521$) and springiness which was not affected by the MTGase/HPP treatments ($p = 0.0669$).

### 3.2. TPA analysis for samples set at 25 °C and with no HPP treatment

A comparison of TPA parameters for samples without pressure treatment, obtained from fish paste without MTGase and gels with added MTGase and set at 25 °C for 2 h, is shown in Fig. 1. Gels containing MTGase did not show fracturability and had higher values of hardness, chewiness, springiness and cohesiveness than did the control. These results suggest that MTGase induced crosslinking of adjacent myofibrillar proteins during incubation, at 25 °C, of fish paste obtained from arrowtooth flounder. This effect is well known in other species such as silver carp (*Hypophthalmichthys molitrix*) (Téllez-Luis, Uresti, Ramírez, & Vázquez, 2002) or Mexican flounder (*Cyclopsetta chittendenni*) (Téllez-Luis, Ramírez, & Vázquez, 2004).

### 3.3. TPA analysis for samples set at 25 °C subjected to HPP treatment

TPA parameters for fish gels produce from fish paste subjected to MTGase/HPP treatments, followed by

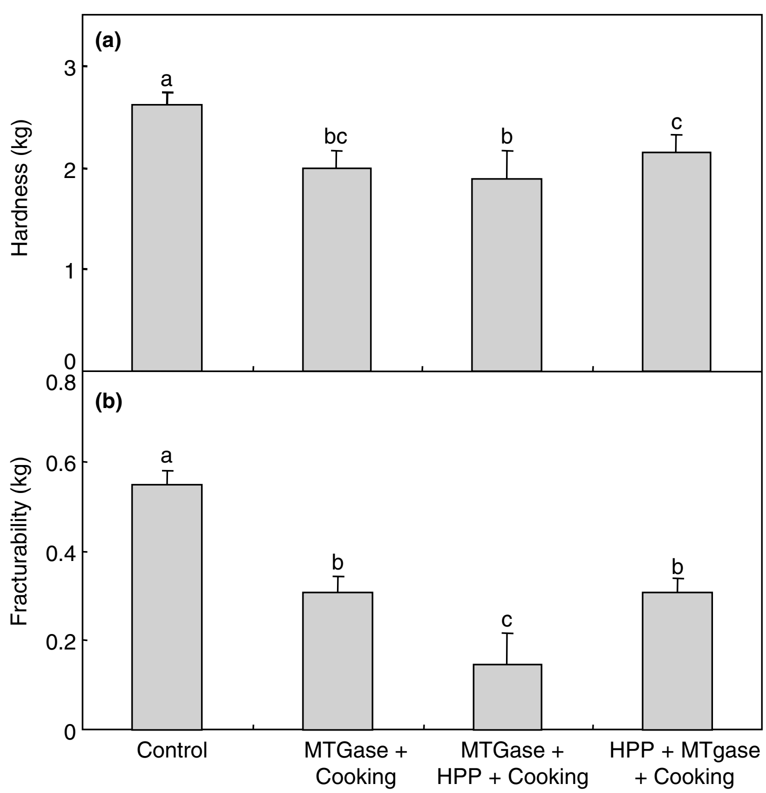


Fig. 4. Effect of MTGase and HPP on the hardness and fracturability of heat-induced gels from fish paste from arrowtooth flounder (*Atheresthes stomias*) mince incubated at 40 °C for 30 min. Different letters indicate differences ($p < 0.05$) between treatments (columns).

cooking at 90 °C for 15 min after setting at 25 °C for 2 h are summarized in Figs. 2 and 3. Hardness varied from 2.4 to 2.9 kg, fracturability ranged from 0.49 to 0.61 kg, springiness from 0.36 to 0.48, cohesiveness from 0.17 to 0.23 and chewiness from 0.16 to 0.33 kg. The control gel and the samples set with added enzyme (MTGase + cooking) showed no differences in TPA parameters ($p < 0.05$) indicating that MTGase was unable to increase the mechanical properties of gels. Fish gels, obtained from pastes with added MTGase set at 25 °C and then pressure-treated (MTGase + HPP + cooking), showed the same values of TPA parameters as control gels. However, fish gels containing added MTGase and pressure-treated before setting at 25 °C (HPP + MTGase + cooking), showed stronger mechanical properties than did control gels. These results indicate that HPP modified protein structure, allowing MTGase to induce a stronger structure, thus improving texture attributes. This modification of protein structure by HPP, previously reported by Uresti et al. (2004a, 2004b), has been associated with a pressure-gelling mechanism different from that induced by heating. This observation is consistent with scanning electronic microscopy studies reported in the literature (Fernández-Martín et al., 1998; Borderías et al., 1997).

### 3.4. TPA analysis for samples set at 40 °C subjected to HPP treatment

TPA parameters for fish gels obtained by setting fish pastes at 40 °C for 30 min are summarized in Figs. 4 and 5. Hardness varied from 1.9 to 2.6 kg, fracturability ranged from 0.14 to 0.55 kg, springiness from 0.28 to 0.43, cohesiveness from 0.17 to 0.21 and chewiness from 0.11

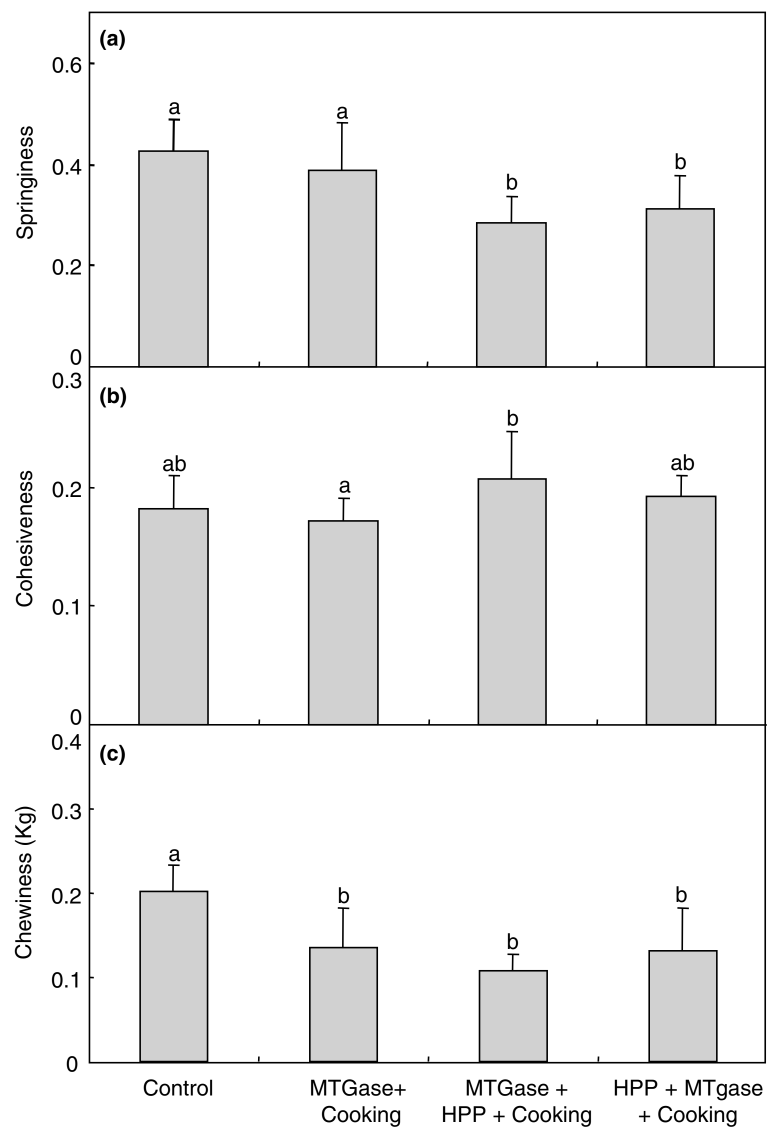


Fig. 5. Effect of MTGase and HPP on the springiness, cohesiveness and chewiness of heat-induced gels from from fish paste from arrowtooth flounder (*Atheresthes stomias*) mince incubated at 40 °C for 30 min. Different letters indicate differences ($p < 0.05$) between treatments (columns).

to 0.20 kg. Control fish gels showed the highest values of hardness, fracturability, springiness and chewiness ($p < 0.05$) of all four treatments studied. The cohesiveness parameter was very low in all gels (<0.2) and showed no differences between treatments. These results showed that, in fish gels set at 40 °C, there was no evidence of protein crosslinking (setting phenomenon) induced by the added MTGase.

Proteolytic enzymes hydrolyze myosin during heating, negatively affecting the mechanical properties of fish gels (Uresti et al., 2004a, 2004b). Restructured arrowtooth flounder with heat treatments similar to those reported in this work were recently studied by Uresti et al. (2004a). These authors observed that TPA values for control samples and fish gels incubated at 40 °C for 30 min, to induce setting by endogenous TGase, did not differ and showed no evidence of proteolytic degradation of myofibrillar proteins. The decrease in TPA values found in this work for samples set at 40 °C and subjected to MTGase/HPP treatments might be associated with a higher proteolytic activity in this batch of arrowtooth flounder. This may also explain why, in this study, samples incubated at 60 °C were totally liquefied while in previous work it was feasible to prepare gels for determination of TPA attributes.

## 4. Conclusions

The addition of MTGase improved the mechanical properties of arrowtooth flounder paste set at 25 °C for 2 h, indicating the presence of protein crosslinking. However, heat-induced gels from arrowtooth flounder paste set at 25 °C and containing MTGase did not show an increase in mechanical properties. Fish paste treated at 600 MPa for 5 min had a modified protein structure, allowing MTGase to improve mechanical properties of heat-induced gels previously set at 25 °C for 2 h. Samples set at 40 °C showed a decrease in the mechanical properties of gels and samples incubated at 60 °C liquefied totally. The results obtained indicate that HPP and MTGase can be used to prepare gels from arrowtooth flounder flesh with improved mechanical properties when set at 25 °C.

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