Mechanical and functional properties of beef products obtained using microbial transglutaminase with treatments of pre-heating followed by cold binding

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ABSTRACT

Beef proteins are considered non-setting proteins and usually gels obtained by adding of microbial transglutaminase are obtained by cooking directly the solubilized paste. The aim of this work was to determine the effect of pre-heating treatments on the mechanical properties of restructured beef gels treated with microbial transglutaminase (MTG). The effect of cooling (cold binding) the solubilized pastes after the pre-heating treatments was also studied. The restructured beef gels were obtained by adding 0.3% MTG or 0% MTG (control). Three pre-heating temperatures (40, 50 or 60 °C) for 30 or 60 min were studied, followed by heating at 90 °C for 15 min. Control samples without pre-heating were also prepared. Cold binding was studied by holding pre-heated gels at 4 °C for 12 h before heating at 90 °C for 15 min. Changes in mechanical properties (texture profile analysis and puncture test), color attributes, expressible water and cooking loss were determined. Results indicated that the better mechanical properties can be obtained by pre-heating beef pastes at 50 °C for 30 min with minimal effect on color, expressible water and cooking loss when 0.3% of MTG is added. It was concluded that there were no practical advantages by pre-heating the gels for 60 min. Cold binding did not improve the mechanical properties of beef gels.

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1. Introduction

The meat and poultry industries are always searching for alternatives to utilize low-value cuts and trimmings by transforming them into marketable products with added value. Frequently, restructuring is chosen as a good alternative for this purpose (Dondero, Figueroa, Morales, & Curotto, 2006). Several practices such as washing of mechanically deboned chicken meat (Perlo, Bonato, Teira, Fabre, & Kueider, 2006), the use of microbial transglutaminase (MTG) as a cold binder (Cofrades, Ayo, Serrano, Carballo, & Jiménez, 2006) or improving the mechanical properties of meat products by cooking (Dondero et al., 2006) have been adopted by the meat and poultry industries from the fish industry.

The MTG enzyme induces the cross-linking of adjacent proteins forming isopeptidic covalent bonds by catalyzing an acyl transfer reaction between γ-carboxyamide groups of glutamyl residues and the ε-amino group of lysine residues, forming the ε-(γ-glutamyl) lysine cross-link (Kumazawa, Seguro, Takamura, & Motoki,

1993; Seki, Nozawa, & Shaowei, 1998). The potential use of MTG to improve mechanical properties of restructured products comes from the surimi industry. Fish muscle contains a calcium dependent endogenous transglutaminase which has good activity at the temperature of myofibrillar protein denaturation. Thus when solubilized fish pastes are incubated at temperatures lower than 40 °C, a low temperature gelling phenomenon called setting or "suwari" is obtained and mechanical properties of the cooked products are enhanced. Optimal temperature for setting depends on both the temperature of protein denaturation/aggregation (to expose the buried protein residues) and the temperature for optimal activity of the endogenous transglutaminase. Protein denaturation of fish muscle is highly dependent on habitat temperature. Fishes inhabiting cold water produce better gels when setting is obtained at temperatures lower than 25 °C. On the contrary, warm water species present better attributes at high-temperature setting (over 35 °C) (Kamath, Lanier, Foegding, & Hamman, 1992; Lee & Park, 1998; Ramirez, Rodriguez-Sosa, Morales, & Vazquez, 2000; Ramirez, Santos, Morales, Morrisey, & Vazquez, 2000; Torres-Arreola, Pacheco-Aguilar, Sotelo-Mundo, Rouzaud-Sández, & Ezquerra-Brauer, 2008).

Meat and poultry are considered non-setting proteins. Consequently, a pre-heating treatment to allow optimal cross-linking has not been considered, the products usually being obtained

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directly by cooking (Castro-Briones et al., 2009). Although several studies using MTG in beef or poultry products have used incubating temperatures in the range of 40–45 °C, this treatment might not be considered as optimum because poultry and beef myosin denature at 55 °C (Samejima, Egelandsdal, & Fretheim, 1985). Dondero et al. (2006) reported that beef gels showed better mechanical properties when incubated at 55 °C than at 45 °C or 4 °C. Recently, our results showed that beef gels containing 0.3% of MTG incubated at 50 °C for 30 min, showed better mechanical properties than control gels cooked directly without incubation or gels incubated at 40 or 60 °C for 30 min (Castro-Briones et al., 2009).

MTG is considered as a useful binding agent for fresh beef. This allows the development of new meat products with potential attraction for consumers. This method is called cold binding and induces the cross-linking of solubilized myofibrillar proteins during chilled storage. Kolle and Savell (2003) studied this process using MTG and casein. The cold binding implies an aggregation of the myofibrillar proteins without the phenomenon of denaturation/aggregation induced by heat. In the cold binding, the aggregation is due to the effect of MTG.

The objective of this work was to determine the effect of a pre-heating treatment (followed or not followed by cold binding) on the mechanical properties of beef gels obtained with MTG.

2. Materials and methods

2.1. Raw materials

Whole pieces of "round tip steak" were obtained from a local store in Reynosa (Tamaulipas, Mexico) which only sells meat from Federal Inspection slaughterhouses. The cuts were transported to the laboratory, stored in ice and trimmed and cleared of excess of fat and connective tissue. Then the meat was ground using a meat grinder (Model 10022, Torrey, Monterey, NL, Mexico) with a 5-mm plate.

Microbial transglutaminase was kindly supplied by NUTRER S.A de C.V. (Mexico). The activity of the enzyme was reported by the producer as 100 U/g and the composition of the product was described by the provider as maltodextrin-sodium caseinate (99%) and transglutaminase (1%).

2.2. Preparation of gels

The ground meat was chopped and solubilized by adding 2.0% (w/w) NaCl for 10 min in a cutter with a capacity of 5.51 kg (Hobart model 84145, Hobart Inc., Troy, OH). At the same time, 0.3% (w/w) of powdered MTG was added (untreated gels without the enzyme were also prepared). The mixture was stuffed with a hand-operated stainless steel stuffer (model E17–1, Polinox S.A) in capped stainless steel tubes (18-cm length and 1.87-cm internal diameter) with a capacity of approximately 110 g. The tubes were lubricated with edible oil to facilitate gel extraction.

2.3. Thermal treatments

The tubes were incubated in water at 40, 50 or 60 °C for 30 or 60 min, followed by cooking in water at 90 °C for 15 min. The cooking at 90 °C was to allow a quick inhibition of the transglutaminase. The times used in these treatments (30 min for setting and 15 min for cooking) are usual in this kind of process. After cooking, the tubes were immediately removed, placed in a water bath and cooled at 4–5 °C for 30 min. Cold binding restructured gels were obtained by storing at 4 °C for 12 h the pre-heated tubes (incubated in water at 40, 50 or 60 °C for 30 or 60 min). Control gels without pre-heating were also obtained by heating at 90 °C for 15 min. Immediately after chilled storage, the tubes were cooked

in water at 90 °C for 15 min, placed in a water bath and cooled at 4–5 °C for 30 min. All the gels were removed from the tubes and were stored overnight at 4 °C in polystyrene bags prior to testing.

2.4. Mechanical properties of gels

Mechanical properties were measured using a texturometer TA-XT2i Stable Micro Systems Texturometer (Vienna Court, England). Gel samples were cut into small cylinders (2.5 x 1.87 cm) and kept in polyethylene bags for 1 h at 4 °C to avoid dehydration before analysis.

Texture profile analysis (TPA) was carried out using a cylindrical aluminum probe (P/50) of 50 mm of diameter. Samples were compressed to 75% of the original height, using a compression speed of 60 mm/min. Fracturability, hardness, springiness, cohesiveness, and chewiness were determined. Fracturability was reported as the force at the first fracture point. Hardness was determined as the force at the maximum height of the first compression. Cohesiveness was calculated as the ratio of the areas of the second and the first compression. Springiness was calculated as the height on the second compression divided by the height of the first compression. Chewiness was calculated by multiplying together hardness, cohesiveness, and springiness (Anton & Luciano, 2007). Six samples were analyzed for each treatment.

A puncture test was performed by compressing samples to 75% of initial height using a compression speed of 60 mm/min with a 12 mm spherical probe (P/0.5 s). The samples were penetrated at the center. Breaking force (N), deformation (cm), and penetration work (N · cm) were calculated. Six samples were analyzed for each treatment.

2.5. Expressible water of gels

The expressible water (E_W) for each treatment was measured. Samples of 3 g (±0.2 g) of gels were weighed and put into two layers of filter paper. Samples were placed at the bottom of 50 mL centrifuge tubes and centrifuged at 10000g for 15 min at 15 °C. Immediately after centrifugation, the samples were weighed and the E_W was calculated as follows:

$$E_W = \frac{W_i - W_f}{W_i} \cdot 100$$

Where W_i is the initial weight of sample and W_f is the final weight of sample.

2.6. Color attributes

Spectral reflectance of restructured beef gels was determined using a portable colorimeter (HunterLab MiniScan XE Plus spectrocolorimeter model 45/0-L, Hunter Assoc., Reston, Va., USA) calibrated against black and white tiles. CIE L^*, a^*, and b^* values, chroma ($[a^{*2} + b^{*2}]^{1/2}$), and hue angle (arc tan b^*/a^*) were calculated based on illuminate C and the 2° standard observer.

2.7. Cooking loss

Cooking loss was determined by weighing the solubilized beef paste introduced into the tubes before cooking and weighing the final cooked gels extracted from tubes. Cooking loss was expressed as a percentage of the initial weight. Three replicates of each treatment were measured.

2.8. Statistical analysis

Data were analyzed by one-way ANOVA (Statgraphics Ver. 5, Software Publishing Corporation, Bitstream Inc, Cambridge, MA,

USA). Differences between mean values were established using the least significant difference (LSD) multiple range test ($P \leqslant 0.05$). One-way ANOVA was conducted to determine differences between pre-heating conditions for gels obtained with similar treatment (pre-heating or pre-heating plus cold binding).

3. Results and discussion

This study reports the effect of pre-heating the solubilized beef pastes containing 0.3% of MTG or without enzyme before inducing a cold binding at 4 °C. Gels were incubated at 40, 50 or 60 °C for 30 min or 60 min (before cooking at 90 °C for 15 min). Changes in the mechanical properties (puncture test and texture profile analysis), color, extruded water and cooking loss were evaluated. One-way analysis was conducted to determine differences between gels obtained with different pre-heating treatment in each process: pre-heating or pre-heating plus cold binding. For comparative purposes, control gels without pre-heating were obtained by cooking at 90 °C for 15 min.

3.1. Puncture test

The puncture test values of pre-heated gels are shown in Fig. 1. The breaking force (BF) value of control gels obtained without MTG and without pre-heating treatment was 19.7 N. Adding 0.3% MTG did not increase the breaking force of gels cooked directly without the pre-heating treatment. Pre-heating treatment induced a significantly ($P \leqslant 0.05$) increase in the BF values. Gels incubated at 50 °C for 30 min showed the highest values of BF (37.4 N). This BF value was almost the double of the BF value of the control gel. Only gels pre-heated at 40 °C for 60 min showed a higher BF than gels pre-heated for 30 min. Gels incubated at 50 or 60 °C showed a decrease in the BF values as compared with gels incubated for 30 min. The control gel without MTG showed the lowest value of deformation. This parameter was increased in the gel containing MTG cooked directly without pre-heating. The highest value of deformation was obtained by pre-heating gels at 50 °C for 30 min or by pre-heating gels at 40 or 50 °C for 60 min. The penetration work parameter (WP) showed a similar behavior to BF. The gel obtained by pre-heating at 50 °C for 30 min showed the highest value of WP (420 N · cm) which is double the value obtained for the control gel. Gels incubated at 40 °C for 60 min showed higher values ($P \leqslant 0.05$) of puncture test parameters than gels incubated for 30 min at the same temperature. The gels incubated at 50 or 60 °C for 60 min had significantly ($P \leqslant 0.05$) lower values than gels incubated at the same temperatures for 30 min.

The effect of cold binding treatment on the puncture test parameters of beef gels is shown in Fig. 2. The control gels obtained without MTG or pre-heating showed the lowest values of breaking force, deformation and penetration work. The gels obtained with MTG but without the pre-heating treatment showed values of the puncture test parameters that were not different from control gels. Pre-heated gels showed higher values of puncture test parameters than non pre-heated gels. However, gels pre-heated at 50 °C for 30 or 60 min showed higher values of puncture test parameters (Fig. 1) than the same gels stored in refrigeration for 12 h (cold binding gels). On the other hand, the gels stored in refrigeration for 12 h after pre-heating at 60 °C for 60 min showed higher values in all the puncture test parameters than gels obtained only by pre-heating at 60 °C for 60 min.

3.2. TPA

The effect of pre-heating treatments on the fracturability and hardness parameters are shown in Fig. 3. The control gel without

MTG or pre-heating treatment showed the lowest values of fracturability (36.7 N) and hardness (87.9 N). Control gels containing 0.3% MTG but obtained by cooking directly at 95 °C without pre-heating showed a slight but significantly ($P \leqslant 0.05$) increase in both TPA parameters, fracturability and hardness. The gels obtained by pre-heating at 40–60 °C for 30 or 60 min showed significantly ($P \leqslant 0.05$) higher values of fracturability and hardness than gels obtained without the pre-heating treatment. The gels incubated at 50 °C for 30 or 60 min showed almost twice the values of fracturability and hardness compared to the control.

Gels incubated at 40 °C for 60 min showed significantly ($P \leqslant 0.05$) higher values of fracturability and hardness than gels incubated for 30 min at the same temperature. The time of incubation did not affect the mechanical properties of gels incubated at 50 °C, but gels incubated at 60 °C for 60 min had significantly ($P \leqslant 0.05$) lower values than gels incubated at the same temperature for 30 min.

The values of fracturability and hardness for gels obtained after the cold binding treatment are shown in Fig. 4. The behavior of the gels obtained by storing in refrigeration the pre-heated gels was similar to the behavior of the gels cooked directly after the pre-heating treatment (Fig. 3). The control gel with 0.3% MTG and obtained without pre-heating treatment showed slightly but significantly higher values of fracturability and hardness than control gel obtained without MTG or the pre-heating treatment. The gels obtained by the pre-heating treatments showed higher values of hardness and fracturability than control gels. The highest values were found in gels pre-heated at 50 °C for 30 or 60 min. Cold binding produced gels with higher values of fracturability ($P \leqslant 0.05$) when the 40 °C pre-heating treatment was applied for 30 or 60 min. However, the cold binding treatment induced lower values of hardness ($P \leqslant 0.05$) in gels incubated at 50 or 60 °C for 30 or 60 min.

The springiness, cohesiveness, and chewiness parameter of pre-heated gels is shown in Table 1. The springiness was not different ($P \leqslant 0.05$) between the control gels obtained without MTG nor pre-heating treatments (0.743) than controls obtained with 0.3% MTG but without pre-heating (0.754). Springiness was significantly higher ($P \leqslant 0.05$) in gels incubated at 40–60 °C for 30 min or 60 min than control gels, varying from 0.824 to 0.857. There was no significant difference in the springiness parameter of gels obtained with any of the pre-heating treatments. The cohesiveness of all the gels was very low in all treatments varying from 0.215 to 0.256. There was no significant difference in the cohesiveness parameter of gels obtained by any of the treatments used in this study. The chewiness was not different ($P \leqslant 0.05$) between the control gels obtained without MTG and without pre-heating treatments (1.483) and controls obtained without pre-heating but with 0.3% MTG (1.504). Gels obtained with the pre-heating treatment had higher chewiness ($P \leqslant 0.05$) than control gels. Gels pre-heated at 50 °C for 30 or 60 min, showed the highest values of chewiness, almost double the value of control gels. Gels incubated at 40 °C for 60 min showed higher values ($P \leqslant 0.05$) of chewiness than gel incubated at the same temperature for 30 min. Time of incubation of gels pre-heated at 50 or 60 °C did not differ in chewiness.

The changes in the parameters of springiness, cohesiveness, and chewiness of beef gels stored in refrigeration (cold binding treatment) are shown in Table 2. The control gels obtained without MTG and without pre-heating treatments showed lower value ($P \leqslant 0.05$) of springiness (0.746) than control gels obtained without pre-heating but with 0.3% MTG (0.773). Pre-heated gels showed higher values of springiness than control gels, but there was no difference among treatments ($P > 0.05$).

The cohesiveness of all the gels was very low in all treatments varying from 0.224 to 0.250. There was no significant difference
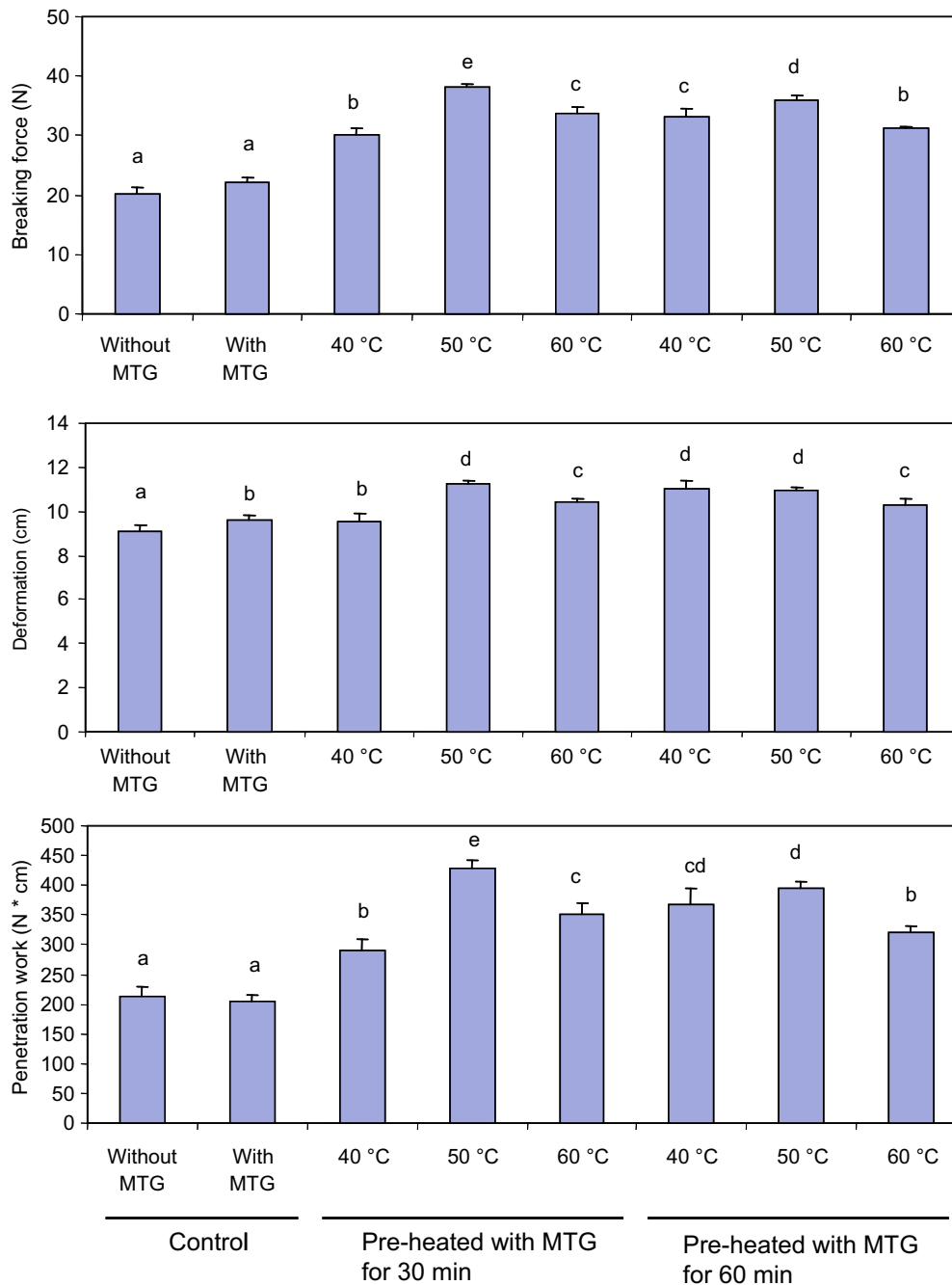
Fig. 1. Effect of pre-heating on the puncture test parameters of beef gels. Mean values of six replicates. Bars indicate standard deviations ($P \leqslant 0.05$). [a,b,c,d,e,f] Different letters indicate differences ($P \leqslant 0.05$) in puncture test parameters among pre-heated gels as affected by treatments.

in the cohesiveness parameter of gels obtained in any of the treatments used in this study.

Chewiness was increased by adding MTG to the control gels obtained without pre-heating treatment. The pre-heating treatment increased the chewiness parameter in all gels. The highest value of chewiness was found in gels pre-heated at 50 °C for 30 or 60 min.

No effect of cold binding was observed for the TPA parameters of springiness, cohesiveness or chewiness for any of the treatments obtained in this study.

Recently our group (Castro-Briones et al., 2009) reported that beef gels obtained with 0.3% MTG combined with a pre-heating treatment of 30 min at 40 to 60 °C showed double the higher mechanical properties of beef gels with 0.3% MTG added and

cooked directly without pre-heating treatment. In this study we increased the time of pre-heating to 60 min to determine if this resulted in gels with higher mechanical properties. Results obtained from this study confirm that pre-heating the gels containing MTG induces the formation of stronger protein network structures inducing stronger mechanical properties than cooking directly. However, there were no practical advantages by increasing the incubating time from 30 to 60 min.

In fish processing technology, the setting phenomenon has an optimal of temperature and time. After reaching the optimal conditions a decrease in the mechanical properties was observed for both endogenous transglutaminase and MTG induced fish gels (Ramirez et al., 2000; Ramirez, Rodriguez-Sosa, Morales, & Vazquez, 2003). This behavior agrees with the observed in beef muscles,
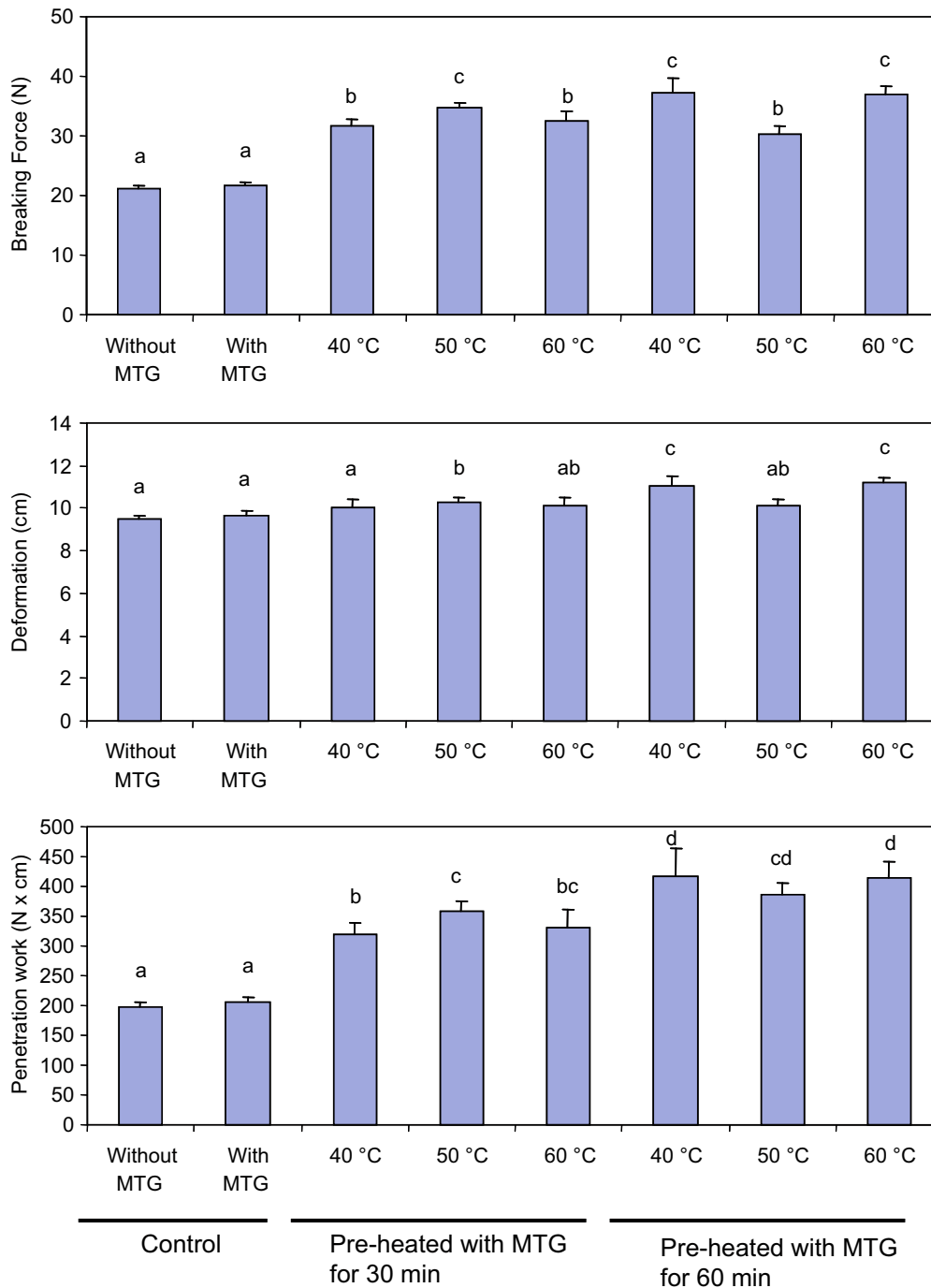
Fig. 2. Effect of pre-heating followed by cold binding on the puncture test parameters of beef gels. Mean values of six replicates. Bars indicate standard deviations ($P \leqslant 0.05$).
[a,b,c] Different letters indicate differences ($P \leqslant 0.05$) in puncture test parameters among pre-heated gels as affected by treatments.

after reaching the maximum mechanical properties at 50 °C for 30 min, a decrease in mechanical properties was observed in gels incubates at 60 °C. Increasing the time of incubating was not important in improving the mechanical properties. Similar results were reported previously (Castro-Briones et al., 2009).

Beef muscle proteins are considered non-setting proteins, because they do not form a gel at low temperature (0–40 °C) like fish muscle proteins. However, it is important to realize that the setting phenomenon is caused by the coincidence of two biological phenomena: (1) denaturation of myofibrillar proteins (myosin and actomyosin) and (2) presence and activity of the endogenous transglutaminase. In fish proteins both phenomena have a concordance in the temperature needed for both protein denaturation

and transglutaminase activity (An, Peters, & Seymour, 1996; Velazquez et al., 2007). However, in beef proteins, this concordance does not exist, endogenous transglutaminase has an optimal temperature at 40 °C (Tsukamasa, Miyake, Ando, & Makinodan, 2000) and beef myosin denatures between 40 and 60 °C (Bendall & Restall, 1983). The use of MTG increases the mechanical properties of beef gels, but a pre-heating treatment improves the efficiency of the activity of the enzyme inducing the strengthening of the three-dimensional structure that forms the gels and increasing the mechanical properties of the beef gels (Castro-Briones et al., 2009).

MTG shows activity even during chilling temperatures (under 4 °C). This property is used to bind raw pieces of meat under refrigeration, to produce restructured meat products. This practice is
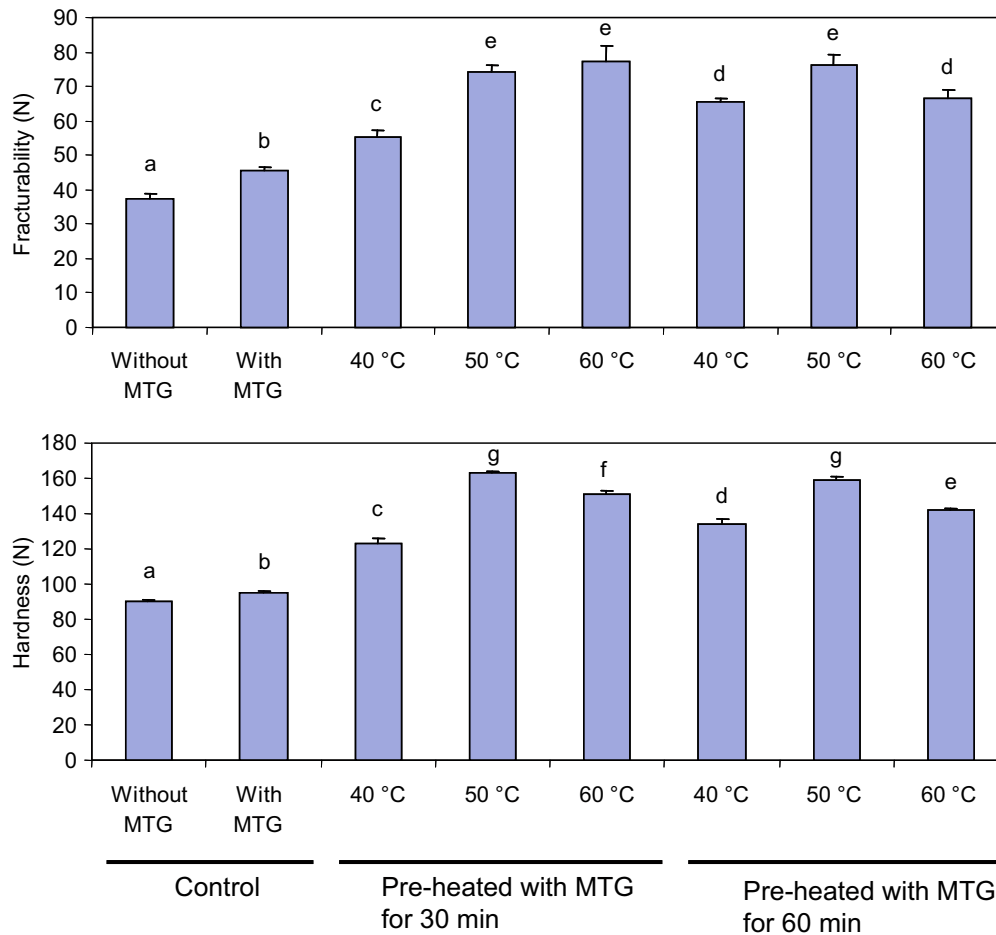
Fig. 3. Effect of pre-heating on the fracturability and hardness parameters of beef gels. Mean values of six replicates. Bars indicate standard deviations ($P \leqslant 0.05$). [a,b,c] Different capital letters indicate differences ($P \leqslant 0.05$) in TPA parameters among pre-heated gels as affected by treatments.

called cold binding. In this work, beef gels were incubated at 40 to 60 °C for 30 or 60 min containing 0.3% MTG and stored under refrigerating temperatures (lower than 4 °C) to determine if pre-denatured proteins allowed production of stronger gels during refrigeration. However, no significant improvement of mechanical properties as affected by the storage of the pre-heated samples containing the enzyme was obtained. This phenomenon could be caused because most of the myosin protein do not remain denatured (refolding) after stopping the pre-heating treatment and chilling the samples (mainly for the 40 and 50 °C treatments) or because most of the myosin protein goes into an irreversibly aggregated state during the pre-heating (mainly for 60 °C) treatments (Nielsen, 1995; O'Kennedy, 2000; Ramirez et al., 2000b). If proteins refold or aggregate the reactive amino acid residues are buried, and MTG activity is inhibited. MTG requires the presence of lysine and glutamic residues to induce a covalent isopeptidic cross-linking of adjacent proteins (Jong & Koppelman, 2002; Kuraishi, Yamasaki, & Susa, 2001). Another explanation could be that the enzyme reaches the maximum of its feasible activity at the level of addition (0.3%) and the conditions used on this study.

The results obtained in this study indicate that beef myofibrillar proteins can show the setting phenomenon when MTG is added if special attention is paid to the pre-heating temperature, because the setting phenomena depends widely on the denaturation temperature of myosin, which induces the exposition of the buried γ-carboxyamide groups of glutamyl residues and ε-amino group of lysine residues during the cross-linking induced by MTG (Ramirez et al., 2003; Cofrades et al., 2006).

3.3. Color attributes

The change in color attributes of beef gels by using MTG and the pre-heating treatment is shown in Tables 3 and 4. There exist slight (less than 10% of control value) but significant changes in the attributes of color ($P \leqslant 0.05$) as affected by treatments used in this study. The Lightness* values varied from 47.19 to 48.94. The control gel obtained without MTG or the pre-heating treatment had an L^* value of 48.42. Only the gels obtained by pre-heating at 50 or 60 °C for 30 min were slightly but significantly different to the control treatment ($P \leqslant 0.05$). The chroma* value varied from 12.23 to 13.79.

The control gel had a C^* value of 13.35. Only the gels obtained by pre-heating at 50 or 60 °C for 30 min were slightly but significantly different to the control treatment ($P \leqslant 0.05$). The Hue* attribute was in the range of 67.88 to 70.38, indicating a yellowish to clear brownish color. However, the low values of L^* and C^*, indicates that gels were appreciated as greyish.

Gels obtained by chilling the pre-heated samples before heating (cold binding) had L^* values from 47.78 to 48.55 and no significant changes ($P \leqslant 0.05$) were observed among treatments. The Chroma* attribute varied from 12.90 to 14.09. Only the gels obtained by pre-heating at 60 °C for 30 min showed a slight but significant higher value of chrome than control. The Hue* attribute varied from 67.15 to 70.5. Only the gels obtained by pre-heating at 60 °C for 30 min and 50 °C for 60 min showed a slight but significant difference of hue to the control. These small differences observed between samples were not large enough to conclude that the treatment modified the color attributes of the samples. The hue
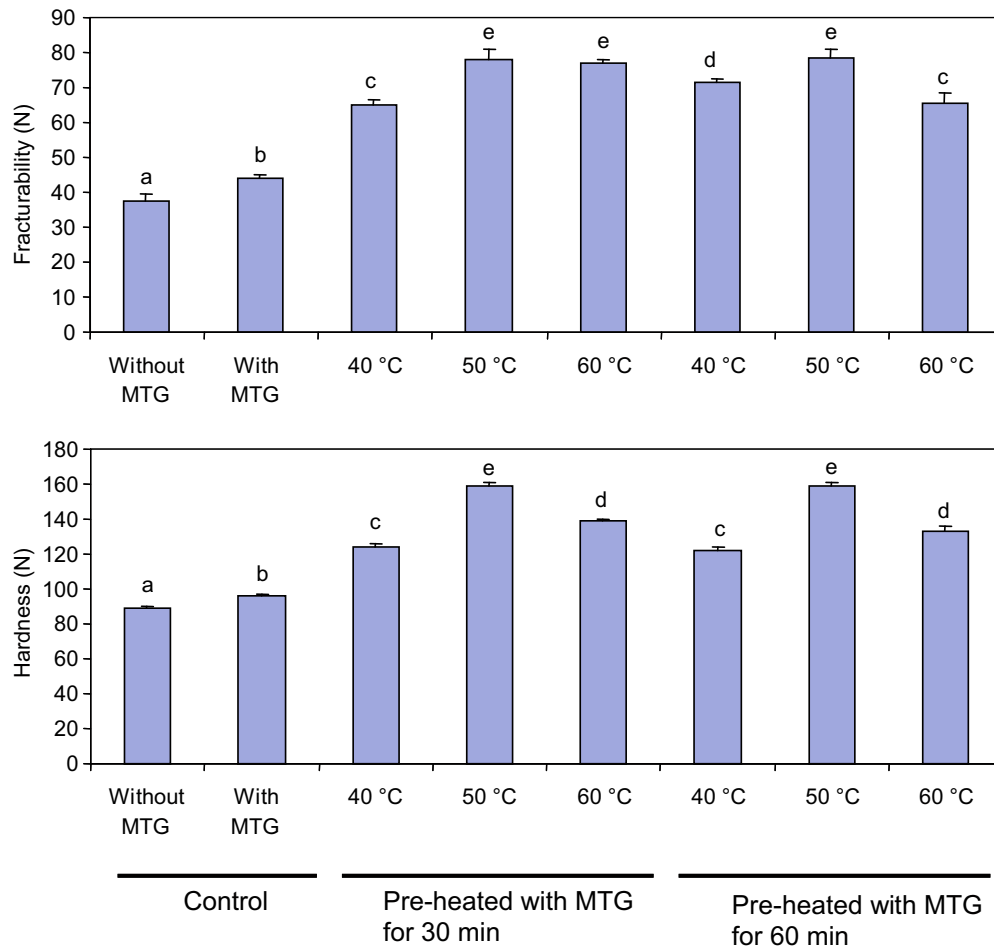
Fig. 4. Effect of pre-heating followed by cold binding on the fracturability and hardness parameters of beef gels. Mean values of six replicates. Bars indicate standard deviations ($P \leqslant 0.05$). [a,b,c] Different capital letters indicate differences ($P \leqslant 0.05$) in TPA parameters among pre-heated gels as affected by treatments.

Table 1
Effect of pre-heating treatments on TPA parameters of the beef gels.

Parameter	Control (no pre-heating)		Pre-heating with MTG for 30 min			Pre-heating with MTG for 60 min		
	Without MTG	With MTG	40 °C	50 °C	60 °C	40 °C	50 °C	60 °C
Springiness	0.743 ± 0.02[a]	0.754 ± 0.01[a]	0.824 ± 0.06[b]	0.843 ± 0.04[b]	0.844 ± 0.05[b]	0.835 ± 0.03[b]	0.857 ± 0.07[b]	0.831 ± 0.10[b]
Cohesiveness	0.215 ± 0.05[a]	0.222 ± 0.03[a]	0.243 ± 0.04[a]	0.251 ± 0.03[a]	0.238 ± 0.03[a]	0.246 ± 0.02[a]	0.256 ± 0.01[a]	0.247 ± 0.09[a]
Chewiness (N)	14.83 ± 0.7[a]	15.04 ± 0.5[a]	24.84 ± 1.0[b]	34.56 ± 0.5[d]	30.36 ± 0.5[c]	27.54 ± 0.4[c]	34.96 ± 0.7[d]	29.30 ± 1.5[c]

[a,b,c]Different letters indicate differences ($P \leqslant 0.05$) in parameters among pre-heated gels as affected by treatments.

Table 2
Effect of pre-heating treatments followed by cold binding at 4 °C for 12 h on TPA parameters of the beef gels.

Parameter	Control (no pre-heating)		Pre-heating with MTG for 30 min			Pre-heating with MTG for 60 min		
	Without MTG	With MTG	40 °C	50 °C	60 °C	40 °C	50 °C	60 °C
Springiness	0.746 ± 0.01[a]	0.773 ± 0.01[b]	0.827 ± 0.03[c]	0.852 ± 0.03[c]	0.823 ± 0.02[c]	0.841 ± 0.04[c]	0.845 ± 0.02[c]	0.819 ± 0.04[c]
Cohesiveness	0.224 ± 0.05[a]	0.237 ± 0.05[a]	0.241 ± 0.06[a]	0.250 ± 0.05[a]	0.242 ± 0.02[a]	0.240 ± 0.03[a]	0.246 ± 0.03[a]	0.247 ± 0.05[a]
Chewiness (N)	14.88 ± 0.6[a]	17.07 ± 0.7[b]	24.72 ± 1.0[c]	33.87 ± 0.9[d]	27.72 ± 0.3[d]	24.58 ± 0.5[c]	33.24 ± 0.7[e]	27.01 ± 0.7[d]

[a,b,c,d,e]Different letters indicate differences ($P \leqslant 0.05$) in parameters among pre-heated gels as affected by treatments.

values indicate the gels had brownish color but the low values of lightness and chroma indicates that gels could be observed as greyish by consumers.

3.4. Expressible water

Changes in the amount of expressible water are shown in Figs. 5 and 6. The amount of expressed water varied from 19.35% to 20.79% in pre-heated gels, and from 18.91% to 21.43% in pre-heated and cold bound gels. The pre-heating treatment induced a slight but significant increase ($P \leqslant 0.05$) in the amount of expressible water in some of the pre-heated gels, indicating a reduction in the water holding capacity (WHC). This behavior was observed also in the cold bound gels. Although there was not an identified pattern for this behavior in gels, as affected by the time or the temperature of pre-heating, all the samples pre-heated at 60 °C for 30 or

Table 3
Effect of pre-heating treatments on color attributes of the beef gels.

Parameter	Control (no pre-heating)		Pre-heating with MTG for 30 min		
	Without MTG	With MTG	40 °C	50 °C	60 °C
Ligthness[*]	48.42 ± 0.29[a]	48.69 ± 0.33[a]	48.40 ± 0.13[a]	47.19 ± 0.68[b]	48.94 ± 0.22[c]
a[*]	5.02 ± 0.13[a]	4.77 ± 0.08[b]	4.61 ± 0.05[c]	4.39 ± 0.11[d]	4.95 ± 0.06[a]
b[*]	12.36 ± 0.13[a]	12.69 ± 0.28[a,b]	12.44 ± 0.12[a]	11.40 ± 0.70[c]	12.87 ± 0.16[b]
Chroma[*]	13.35 ± 0.15[a]	13.56 ± 0.25[a]	13.26 ± 0.13[a]	12.23 ± 0.67[c]	13.79 ± 0.17[b]
Hue[*]	67.88 ± 0.50[a]	69.35 ± 0.61[b]	69.63 ± 0.16[b]	68.63 ± 1.21[a,b]	68.97 ± 0.12[b]

[a,b,c,d]Different letters indicate differences ($P \leqslant 0.05$) in parameters among pre-heated gels as affected by treatments.

Table 4
Effect of pre-heating treatments followed by cold binding at 4 °C for 12 h of the beef gels on color attributes of the beef gels.

Parameter	Control (no pre-heating)		Pre-heating with MTG for 30 min			Pre-heating with MTG for 60 min		
	Without MTG	With MTG	40 °C	50 °C	60 °C	40 °C	50 °C	60 °C
Ligthness[*]	48.39 ± 0.20[a]	48.20 ± 0.27[a]	48.05 ± 0.60[a]	48.14 ± 0.28[a]	48.14 ± 0.35[a]	48.55 ± 0.58[a]	47.78 ± 0.55[a]	47.84 ± 0.32[a]
a[*]	4.73 ± 0.05[a]	4.66 ± 0.23[a]	4.86 ± 0.13[a]	4.79 ± 0.17[a]	5.47 ± 0.18[b]	4.92 ± 0.13[a]	4.39 ± 0.22[a]	4.89 ± 0.98[a,b]
b[*]	12.34 ± 0.30[a]	12.81 ± 0.26[a]	11.95 ± 0.35[a]	12.48 ± 0.10[a]	12.98 ± 0.12[b]	12.72 ± 0.27[a,b]	12.38 ± 0.26[a]	12.42 ± 0.18[a]
Chroma[*]	13.22 ± 0.28[a]	13.65 ± 0.26[a]	12.90 ± 0.36[a]	13.37 ± 0.13[a]	14.09 ± 0.16[b]	13.64 ± 0.30[a]	13.14 ± 0.31[a]	13.35 ± 0.20[a]
Hue[*]	68.95 ± 0.57[a]	70.00 ± 1.02[a]	67.82 ± 0.52[a]	69.02 ± 0.66[a]	67.15 ± 0.61[b]	68.85 ± 0.19[a]	70.50 ± 0.73[c]	68.49 ± 0.20[a]

[a,b]Different letters indicate differences ($P \leqslant 0.05$) in parameters among pre-heated gels as affected by treatments.
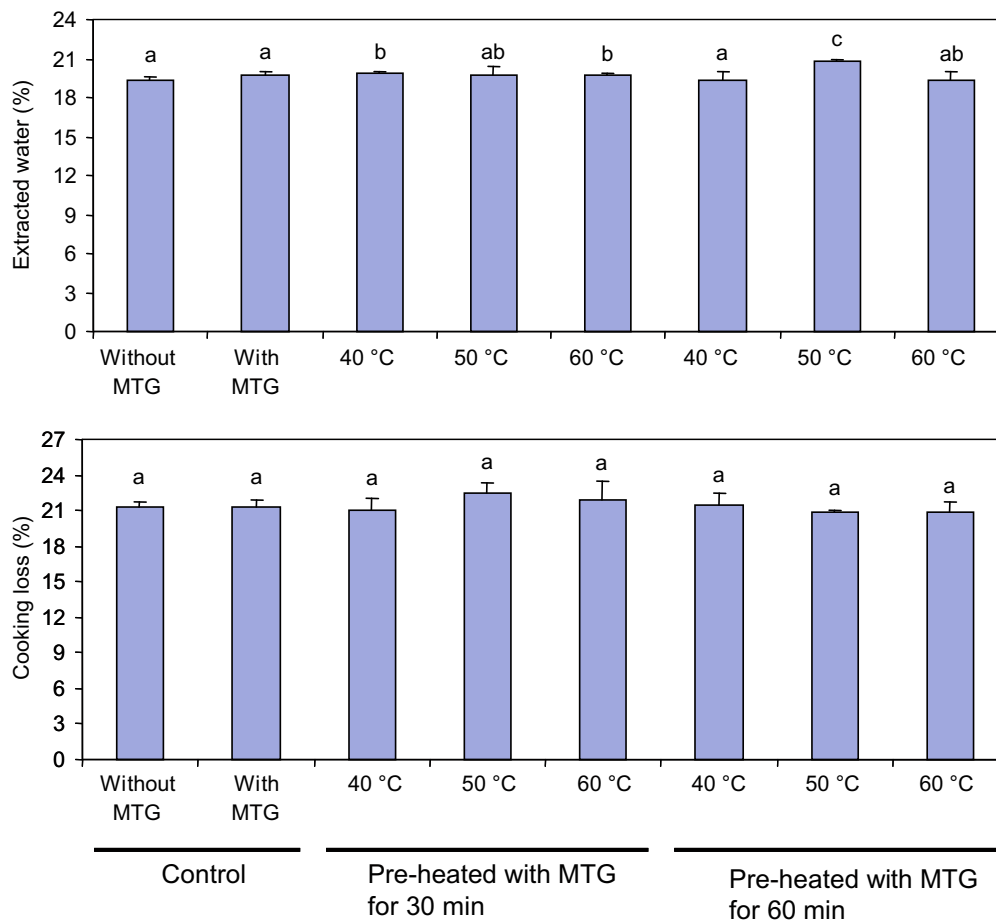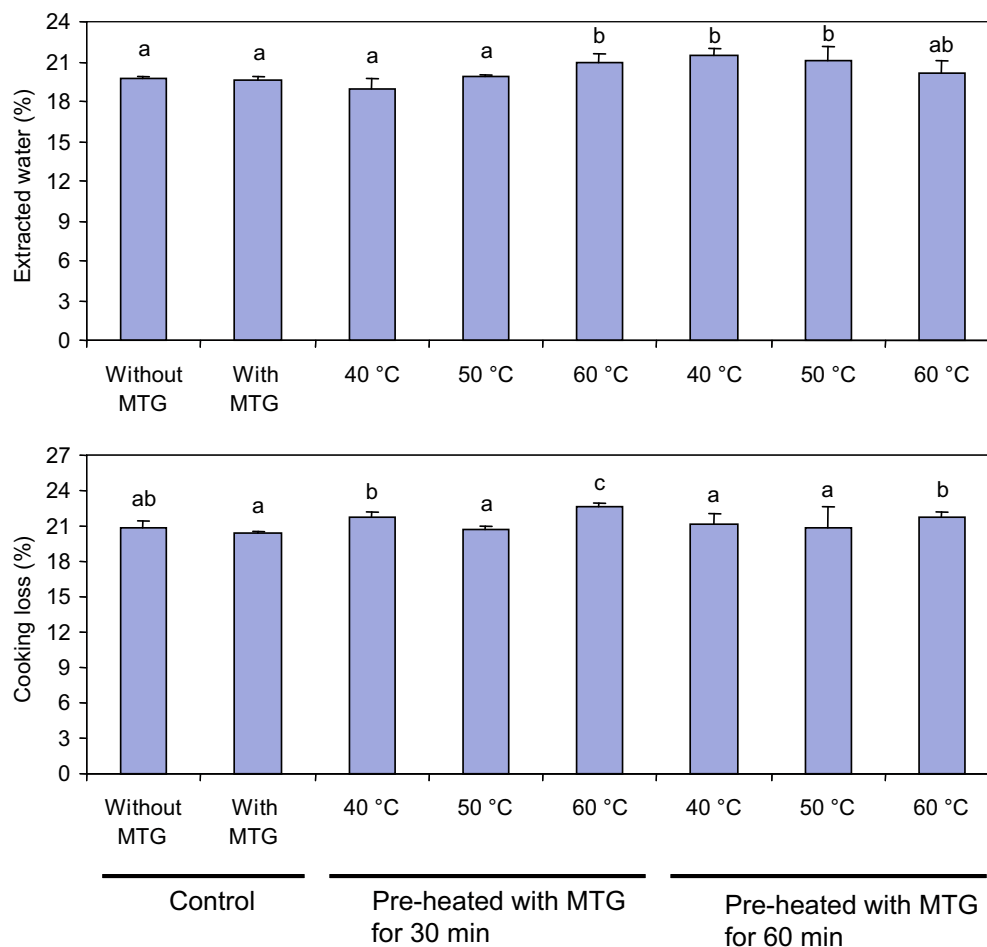

Fig. 5. Effect of pre-heating on the amount of extracted water and cooking loss parameters of beef gels. Mean values of six replicates. Bars indicate standard deviations ($P \leqslant 0.05$). [a,b,c] Different capital letters indicate differences ($P \leqslant 0.05$) in the extracted water and cooking loss parameters among pre-heated gels as affected by treatments.

60 min showed a lower WHC. This decrease in WHC capacity for gels incubated at 60 °C for 30 min has been reported previously by our group (Castro-Briones et al., 2009). Cofrades et al. (2006) also found a decrease in WHC of gels with MTG.

3.5. Cooking loss

Changes in the amount of cooking loss (CL) are shown in the Figs. 5 and 6. The amount of CL varied from 20.82% to

Fig. 6. Effect of pre-heating followed by cold binding on the amount of expressed water and cooking loss parameters of beef gels. Mean values of six replicates. Bars indicate standard deviations ($P \leqslant 0.05$). [a,b,c] Different capital letters indicate differences ($P \leqslant 0.05$) in amount of expressed water and cooking loss parameters among pre-heated gels as affected by treatments.

21.86% in pre-heated gels, and from 20.33% to 22.61% in pre-heated and cold bonded gels. The pre-heated gels did not show a change in the CL compared with the control gel ($P > 0.05$). In the chilled samples (cold binding treatment) only the gels pre-heated at 60 °C for 30 min had a slight but significant ($P \leqslant 0.05$) increase in the CL as compared with the control. Pietrasik and Li-Chan (2002) reported a decrease in the cooking loss. Dondero et al. (2006) found that raising the amount of MTG increased the cooking loss of beef gels. Castro-Briones et al. (2009) reported no significant changes by effect of adding MTG or by thermal treatment in the CL of beef gels. Therefore the effect of MTG on the cooking loss of beef gels remains controversial in the literature.

4. Conclusions

The setting phenomena can be induced in myofibrillar beef proteins when an appropriate pre-heating treatment is induced. Optimal conditions for setting beef proteins seem to be near 50 °C for 30 min. The chilled storage of pre-heated beef proteins containing 0.3% MTG for 12 h at 4 °C did not improve the mechanical properties of gels, despite this practice being used to cold bind meat pieces to obtain restructured products. Using of the pre-heating treatment can be useful to improve mechanical properties of the beef gel or to reduce the amount of MTG needed in the process.

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