EFFECT OF SETTING CONDITIONS USING MICROBIAL TRANSGLUTAMINASE DURING OBTENTION OF BEEF GELS

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ABSTRACT

Microbial transglutaminase (MTG) is a food additive widely used to improve the mechanical properties of beef, poultry and fish gels. The aim of this study was to determine the effect of incubation temperature on the mechanical properties of restructured beef gels treated with MTG. The restructured beef gels were obtained by adding 0.0% (untreated) or 0.3% MTG. Three incubation temperatures (40, 50 or 60C) for 30 min were used, followed by cooking at 90C for 15 min. Control samples without incubation were also prepared. Changes in the mechanical properties (texture profile analysis and puncture test), color attributes, expressible water and cooking loss were determined. Results indicated that the maximum mechanical properties can be obtained by incubating beef pastes at 50C for 30 min with minimal effect on color, expressible water and cooking loss when 0.3% of MTG is added.

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PRACTICAL APPLICATIONS

Meat and poultry are considered nonsetting proteins, and consequently, an incubating treatment to allow optimal cross-linking has not been considered, and the products are usually obtained by direct cooking. Our results showed that beef gels containing 0.3% of microbial transglutaminase incubated at 50C showed higher mechanical properties than control gels without incubation. These results could be useful to improve the mechanical properties of beef gels.

INTRODUCTION

The meat industry is adopting several processes from the fish surimi technology. These processes include the washing of mechanically deboned chicken meat for use in nuggets or similar products (Perlo *et al.* 2006), use of microbial transglutaminase (MTG) to improve the mechanical properties of meat products during cooking (Dondero *et al.* 2006) or obtaining restructured products at chilling temperature by inducing the cold setting with MTG (Cofrades *et al.* 2006). However, there is no general agreement on which could be the most appropriated thermal treatment required to obtain the maximum efficiency of the MTG activity in restructured meat or poultry products.

Fish muscle proteins have a unique capability to form a translucent and highly deformable gel after being solubilized with salt when incubated below 40C. This phenomenon is called setting, and depends on the presence and activity of the endogenous transglutaminase (TGase) enzyme (An *et al.* 1996; Velazquez *et al.* 2007). This enzyme is calcium-dependent and 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-linking (Kumazawa *et al.* 1993; Seki *et al.* 1998). The optimal temperature for setting response depends on fish species and habitat temperature. The mechanical properties of gels from fishes in habiting cold water are better when they are obtained using low-temperature setting (lower than 25C), while gels obtained from warm water species present better attributes at high-temperature setting (higher than 35C) (Kamath *et al.* 1992; Lee and Park 1998; Ramírez *et al.* 2000b,c). Setting induced by endogenous TGase is highly dependent on the incubation temperature of solubilized pastes, because this phenomenon depends on both the temperature of protein denaturation/aggregation and the temperature for optimal activity of the endogenous TGase. Endogenous TGase has an optimal temperature below 40C, while commercial MTG has an optimal temperature at 60C. However, both endogenous TGase

and MTG showed similar optimal temperature for inducing setting in tropical fish species (40C), because this temperature is optimal for myosin denaturation. Above this temperature, protein aggregation inhibits the activity of both types of TGase. Reported studies have established that the optimal temperature for TGase activity depends on the temperature of myofibrillar protein denaturation (Ramírez *et al.* 2000b,c, 2003).

The studies dealing with the use of MTG to produce stronger meat restructured products vary widely. Meat and poultry are considered nonsetting proteins, and consequently, an incubating treatment to allow optimal crosslinking by MTG has not been considered, and the products are usually obtained by direct cooking.

Some studies report incubating temperatures in the range of 40–45C, similar to the temperatures used to set fish proteins. However, myosin, considered as the main myofibrillar protein responsible for the mechanical properties of fish gels (Ramírez *et al.* 2000a), denatures at different temperatures in fishes and in mammalian vertebrates. Fish myosin denatures at 40C, while poultry and beef myosin denature at 55C (Samejima *et al.* 1985). Thus, the incubating temperature to expose buried residues should be different. Dondero *et al.* (2006) reported that beef gels showed higher mechanical properties when incubated at 55C than when incubated at 45 or 4C. Myosin from Alaska Pollock, a cold water-inhabiting fish specie denatured from 10C, reaching the first peak at 32.7C, while myosin from white croaker, a warm water-inhabiting fish specie, started to denature at 30C, reaching the maximum temperature at 34.8C. Rabbit myosin denatures at 45.4C (Fukushima *et al.* 2003).

MTG is considered as an efficient but expensive additive for binding muscle proteins to produce restructured products. Thus, there is a need to determine the optimal conditions for MTG activity in order to maximize the activity of the enzyme to obtain the required textural parameters. The activity of MTG requires combining the optimal temperature of myofibrillar protein denaturation previous to protein aggregation with the optimal temperature for MTG activity (Ramírez *et al.* 2000b,c, 2003).

The objective of this work was to determine the effect of temperature of incubation on the mechanical properties of restructured beef gels obtained with MTG.

MATERIALS AND METHODS

Raw Meat

Whole pieces of "round tip steak" were obtained from a local store, which only expends meat from Federal Inspection slaughterhouses. The cuts were

trimmed and cleared from the 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.

MTG

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

Preparation of Restructured Beef Gels

To prepare restructured beef gels, ground meat was chopped for 10 min in a cutter with a capacity of 5.51 kg (Hobart model 84145, Hobart Inc., Troy, OH). The paste obtained was solubilized by adding 2.0% (w/w) NaCl. At the same time, 0.3% (w/w) of powdered MTG was added (untreated gels without the enzyme were also obtained). The mixture was stuffed with a hand-operated stainless steel stuffer (model E17-1, Polinox S.A, Mexico DF, Mexico) in capped stainless steel tubes (18-cm length × 1.87-cm internal diameter) with a capacity of approximately 110 g. The tubes were lubricated with edible oil to facilitate the gel extraction. The tubes were incubated in water at 40, 50 or 60C for 30 min, followed by cooking in water at 90C for 15 min. 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 4–5C for 30 min. All the gels were removed from the tubes and were stored overnight at 4C in polystyrene bags prior to testing.

Mechanical Properties of Restructured Beef Gels

Mechanical properties were measured using a texturometer TA-XT2i Stable Micro System Texturometer (Surrey, U.K.) with gel samples cut in small cylinders (2.5 × 1.87 cm) kept in polyethylene bags for 1 h at 4C to avoid dehydration before analysis.

Texture profile analysis (TPA) was carried out using a cylindrical aluminum probe (P/50) of 50 mm of diameter. The samples were compressed at 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 measured as the ratio of the areas of the second and the first peak. Springiness was calculated as the height of the product on the second compression divided by the height of the first peak. Chewiness was calculated

as hardness × cohesiveness × springiness (Anton and Luciano 2007). Six samples were analyzed for each treatment.

Puncture test was performed by compressing the samples at 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 (kg), deformation (cm) and penetration work (kg × cm) were calculated. Six samples were analyzed for each treatment.

Expressible Water of Restructured Beef 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. The samples were placed at the bottom of 50-mL centrifuge tubes and were centrifuged at 10,000 × g for 15 min at 15C. 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 the sample and W_f is the final weight of the sample.

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) 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.

Cooking Loss

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

Statistical Analysis

Data were analyzed by multifactorial analysis of variance (Statgraphics Ver. 5, Software Publishing Corporation, Bitstream Inc., Cambridge, MA). Differences between mean values were established using the least significant difference multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

This study reports the effect of incubating at 40, 50 or 60C for 30 min (before cooking at 90C for 30 min) the solubilized beef pastes containing 0.3% of MTG or without enzyme on the mechanical properties, color, extracted water and cooking loss. In this work, gels with 0% of MTG are called untreated gels. Gels without incubation are called control gels. Control gels were obtained by cooking at 90C for 30 min without incubation for both enzyme levels (0 and 0.3% of MTG).

TPA

The effect of incubation temperature and MTG on fracturability and hardness of beef gels is shown in Fig. 1. Control gels (without incubation) showed the lowest value of fracturability (10.07 kg). Untreated beef gels (without MTG) incubated at 40, 50 or 60C for 30 min did not show different fracturability values ($P \leq 0.05$) than the samples without incubation. Gels containing 0.3% of MTG without incubation treatment showed a higher value of fracturability (11.56 kg) than the untreated samples ($P \leq 0.05$).

With the same thermal treatment, the gels obtained by adding 0.3% of MTG showed a higher value of fracturability than the untreated gels. Fracturability of gels at 40C for 30 min was not different from the fracturability of gels by direct cooking at 90C for 30 min (control gels). The maximum fracturability was observed in treated gels incubated at 50C.

Hardness showed a similar behavior than fracturability. Gels containing MTG showed higher values of hardness than untreated gels with the same thermal treatment. Maximum hardness was observed in gels incubated at 50C ($P \leq 0.05$).

Changes on springiness, cohesiveness and chewiness are shown in Table 1. Springiness ranged from 0.795 to 0.835 in untreated gels, and from 0.797 to 0.821 in gels added with 0.3% of MTG. Samples containing MTG did not show significant higher values of springiness than untreated gels at the same thermal treatment.

Cohesiveness value was very low in all samples. This parameter ranged from 0.221 to 0.238 in untreated gels, and from 0.249 to 0.267 in gels with MTG. Samples with 0.3% of MTG incubated at 60C showed the highest value of cohesiveness.

Chewiness showed a similar trend of fracturability and hardness. Gels with 0.3% MTG showed higher values of chewiness than untreated gels obtained with similar thermal treatment. The highest value of chewiness ($P \leq 0.05$) was reached with 0.3% MTG and by incubating at 50C for 30 min.

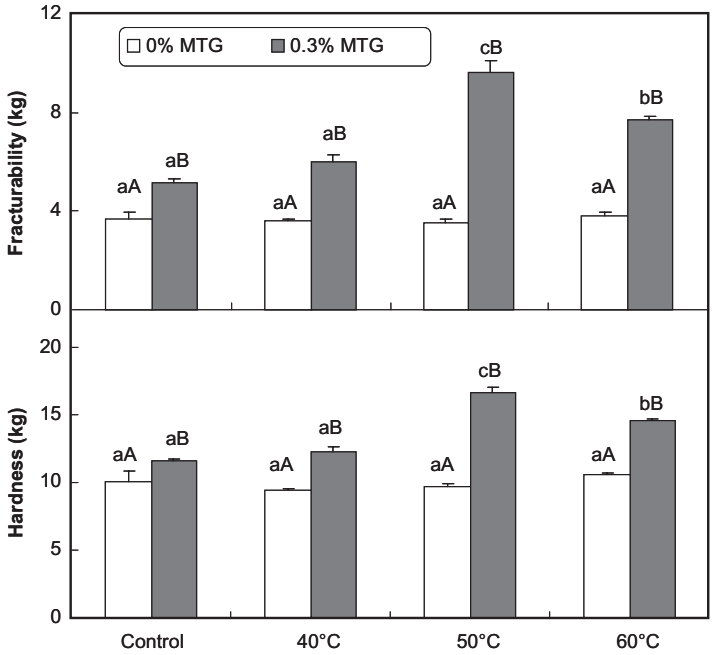

FIG. 1. EFFECT OF INCUBATING TEMPERATURE AND MICROBIAL TRANSGLUTAMINASE (MTG) ADDITION ON THE FRACTURABILITY AND HARDNESS OF TEXTURE PROFILE ANALYSIS PARAMETERS OF BEEF GELS
Mean values of six replicates. Bars indicate SDs.
Different lowercase letters indicate differences ($P \leq 0.05$) in attributes among gels as affected by incubation temperature at the same concentration of MTG.
Different uppercase letters indicate differences ($P \leq 0.05$) in attributes among gels as affected by concentration of MTG at the same temperature.

Puncture Test

Changes on puncture test parameters are shown in Fig. 2. Breaking force (BF) of untreated gels cooked at 90C for 30 min (control) was not different ($P \leq 0.05$) from untreated gels incubated at 40, 50 or 60C for 30 min. The BF value of all gels with MTG was higher than that of untreated gels at the same thermal treatment. The highest BF value was observed in the samples incubated at 50C, and decreased significantly in the samples incubated at 60C.

Deformation and gel strength showed similar behaviors than BF behavior. These have high concordance with the behaviors obtained from TPA parameters of hardness, fracturability and chewiness.

Adding MTG showed a beneficial effect on the mechanical properties of beef gels as reported widely in the literature (Pietrasik and Li-Chan 2002).

TABLE 1.
EFFECT OF INCUBATING TEMPERATURE AND MICROBIAL TRANSGLUTAMINASE (MTG) ON SPRINGINESS, COHESIVENESS AND CHEWINESS OF BEEF GELS

Texture parameter	0% MTG (untreated)				0.3% MTG			
	Control	40C	50C	60C	Control	40C	50C	60C
Springiness	0.805^{aA}	0.795^{aA}	0.801^{aA}	0.835^{bA}	0.814^{bA}	0.813^{bA}	0.821^{bA}	0.797^{aA}
	(0.016)	(0.005)	(0.011)	(0.006)	(0.004)	(0.004)	(0.007)	(0.005)
Cohesiveness	0.238^{cA}	0.231^{bA}	0.221^{aA}	0.221^{aA}	0.253^{aB}	0.249^{aB}	0.253^{aB}	0.267^{bB}
	(0.003)	(0.002)	(0.023)	(0.002)	(0.003)	(0.002)	(0.003)	(0.003)
Chewiness	2.067^{abA}	1.868^{aA}	1.942^{aA}	2.361^{bA}	2.254^{aB}	2.296^{aB}	3.023^{cB}	2.568^{bB}
	(0.203)	(0.052)	(0.091)	(0.031)	(0.046)	(0.069)	(0.097)	(0.041)

Different lowercase letters indicate differences ($P \leq 0.05$) in texture profile analysis (TPA) parameters among gels as affected by incubation temperature at the same concentration of MTG. Values in parentheses indicate the SDs of the means.
Different uppercase letters indicate differences ($P \leq 0.05$) in TPA parameters among gels as affected by concentration of MTG at the same temperature.

Control samples (cooked directly without incubation) with 0.3% MTG showed near to 50% higher mechanical properties than untreated gels (without the enzyme). Incubation of gels with 0.3% MTG increased the mechanical properties in function of the incubation temperature. The samples incubated at 40C showed no differences ($P \leq 0.05$) compared with the samples obtained without incubation. The samples incubated at 50C showed values of fracturability, breaking force and gel strength almost 200% higher than the values of these parameters in control gels. However, gels incubated at 60C showed a decrease in the mechanical properties as compared with gels incubated at 50C.

Untreated beef proteins incubated at 40–60C did not increase the mechanical properties as compared with control samples obtained by direct cooking without incubation. This result indicates the absence of the setting phenomenon induced by the endogenous TGase.

However, treated beef gels with 0.3% of MTG showed higher mechanical properties than control samples. The highest values were observed in samples incubated at 50C. This temperature is higher than the optimal for the endogenous TGase and slightly lower than the optimal for myosin denaturation. But MTG has a higher optimal temperature than endogenous TGase, and 50C is adequate for its use (Wright *et al.* 1977).

The results obtained in this study suggest that setting phenomenon in beef proteins could be induced if special attention is put on the incubation temperature. The effect of incubation temperature on the mechanical properties was more influenced by the denaturation temperature of myosin than by the optimal temperature for the MTG activity. The importance of exposing buried carboxyamide groups of glutamyl residues and ε-amino group of lysine

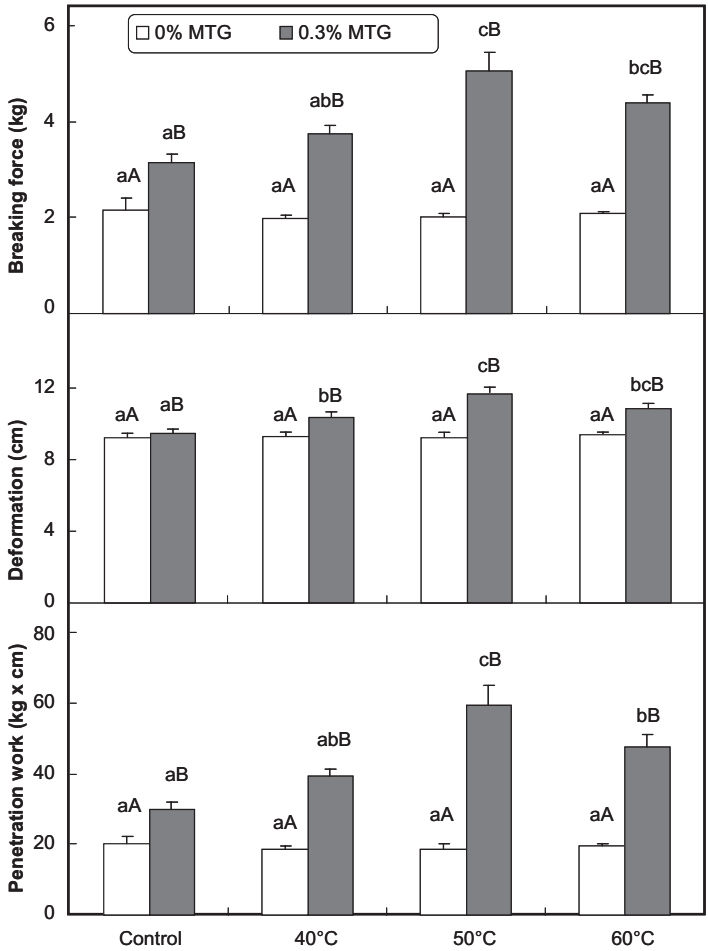

FIG. 2. EFFECT OF INCUBATING TEMPERATURE AND MICROBIAL TRANSGLUTAMINASE (MTG) ADDITION ON PUNCTURE TEST PARAMETERS OF BEEF GELS

Mean values of six replicates. Bars indicate SDs.

Different lowercase letters indicate differences ($P \leq 0.05$) in attributes among gels as affected by incubation temperature at the same concentration of MTG.

Different uppercase letters indicate differences ($P \leq 0.05$) in attributes among gels as affected by concentration of MTG at the same temperature.

residues during the MTG-induced cross-linking has previously been reported (Ramírez *et al.* 2003; Cofrades *et al.* 2006).

The lower increase in the mechanical properties of gels incubated at 40C could be associated with an insufficient denaturation of muscle proteins.

Endogenous TGase has an optimal temperature at 40C (Tsukamasa *et al.* 2000), and MTG is able to induce the cross-linking of adjacent proteins at this temperature, but beef myosin denatures between 40 and 60C (Bendall and Restall 1983). Thus, buried residues were not exposed at 40C, and MTG cannot induce a covalent cross-linking of proteins (Ramírez *et al.* 2000b,c, 2002; Jimenez *et al.* 2005).

Dondero *et al.* (2006) found that beef gels obtained with 0.5% MTG and by incubation at 25, 45 or 60C for 2 h showed higher mechanical properties than gels obtained without incubation by direct cooking. Gels obtained with higher incubating temperature showed higher mechanical properties. In this work, incubating beef gels at 40C for 30 min was not appropriated to improve the mechanical properties of beef gels.

Muscle proteins aggregate irreversibly almost immediately after reaching the maximum denaturation (Nielsen 1995; O'Kennedy 2000; Ramírez *et al.* 2000b). Then, the 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 (Kuraishi *et al.* 2001; Jong and Koppelman 2002). In this study, gels incubated at 60C showed lower mechanical properties than gels obtained at 50C. This behavior seems to be associated with myosin aggregation, which inhibits the MTG activity.

Color Attributes

Changes in color are shown in Table 2. There were no changes in color attributes associated with adding MTG in the samples obtained with the same thermal treatment. However, the thermal treatment affected the color attributes. The highest values of L^* attribute were obtained in the samples incubated at 50C. The highest values of b^* parameter and chrome attribute were obtained in gels incubated at 60C. The parameter a^* and hue attribute were not affected by thermal treatments ($P \leq 0.05$).

Expressible Water

Changes in the amount of extracted water are shown in the Table 3. The amount of extracted water varied from 17.98 to 20.59% in control gels without MTG, and from 17.78 to 20.11% in gels with 0.3% MTG. Untreated gels showed no difference in the amount of extracted water compared to gels with 0.3% MTG when attained with the same thermal treatment ($P \leq 0.05$). However, the thermal treatment modified the amount of extracted water. Gels incubated at 60C showed the highest values of extracted water, which indicates a lower water-holding capacity (WHC). Cofrades *et al.* (2006) found a decrease in WHC of gels with MTG. In this study, addition of MTG did not modify the WHC of beef gels.

TABLE 2.
EFFECT OF INCUBATING TEMPERATURE AND MICROBIAL TRANSGLUTAMINASE (MTG) ON COLOR ATTRIBUTES OF BEEF GELS

Color attributes	0% MTG (untreated)				0.3% MTG			
	Control	40C	50C	60C	Control	40C	50C	60C
L	55.75^{aA}	55.76^{aA}	55.01^{aA}	55.48^{aA}	55.92^{bA}	56.26^{bA}	54.51^{aA}	55.42^{abA}
	(0.39)	(0.53)	(0.39)	(0.14)	(0.59)	(0.57)	(0.36)	(0.24)
*a**	4.42^{aA}	4.13^{aA}	4.15^{aA}	4.37^{aA}	4.03^{aA}	4.31^{aA}	4.51^{aA}	4.56^{aA}
	(0.24)	(0.19)	(0.16)	(0.15)	(0.24)	(0.29)	(0.21)	(0.16)
*b**	12.38^{aA}	12.35^{aA}	12.26^{aA}	12.58^{aA}	12.02^{aA}	12.62^{aA}	12.84^{aA}	12.86^{aA}
	(0.31)	(0.21)	(0.37)	(0.19)	(0.24)	(0.21)	(0.41)	(0.31)
Chrome	13.15^{aA}	13.03^{aA}	12.95^{aA}	13.32^{aA}	12.68^{aA}	13.35^{abA}	13.62^{bA}	13.65^{bA}
	(0.35)	(0.16)	(0.38)	(0.21)	(0.17)	(0.26)	(0.39)	(0.33)
Hue	70.42^{aA}	71.48^{aA}	71.28^{aA}	70.84^{aA}	71.34^{aA}	71.19^{aA}	70.55^{aA}	70.46^{aA}
	(0.69)	(0.98)	(0.48)	(0.45)	(1.38)	(1.02)	(1.04)	(0.43)

Different lowercase letters indicate differences ($P \leq 0.05$) in color attributes among gels as affected by incubation temperature at the same concentration of MTG. Values in parentheses indicate the SDs of the means.
Different uppercase letters indicate differences ($P \leq 0.05$) in color attributes among gels as affected by concentration of MTG at the same temperature.

TABLE 3.
EFFECT OF INCUBATING TEMPERATURE AND MICROBIAL TRANSGLUTAMINASE (MTG) ON THE AMOUNT OF EXTRACTED WATER AND COOKING LOSS

Texture parameter	0% MTG (untreated)				0.3% MTG			
	Control	40C	50C	60C	Control	40C	50C	60C
Extracted water	19.44^{abA}	19.68^{abA}	17.98^{aA}	20.59^{cA}	17.78^{aA}	18.06^{aA}	19.55^{bA}	20.11^{bA}
	(0.35)	(0.74)	(0.09)	(0.87)	(0.27)	(0.66)	(0.18)	(0.37)
Cooking loss	23.08^{aA}	22.53^{aA}	22.58^{aA}	23.74^{aA}	22.84^{aA}	23.54^{aA}	23.19^{aA}	23.81^{aA}
	(0.52)	(1.04)	(0.56)	(1.88)	(1.46)	(0.23)	(0.13)	(1.93)

Different lowercase letters indicate differences ($P \leq 0.05$) in attributes among gels as affected by incubation temperature at the same concentration of MTG. Values in parentheses indicate the SDs of the means.
Different uppercase letters indicate differences ($P \leq 0.05$) in attributes among gels as affected by concentration of MTG at the same temperature.

Cooking Loss

In the literature, the effect of MTG on the cooking loss of beef gels is not clear. Pietrasik and Li-Chan (2002) reported a decrease in the cooking loss, while Dondero *et al.* (2006) found that raising the amount of MTG increased the cooking loss of beef gels. In our study, the cooking loss varied from 22.53

to 23.74% in the samples without MTG, and from 22.84 to 23.81% in the samples with 0.3% MTG. There were no significant changes by effect of adding MTG or by thermal treatment ($P \leq 0.05$).

CONCLUSION

The mechanical properties of beef gels without MTG were not affected by incubating treatment at 40 or 60C for 30 min before cooking at 90C. These results are indicative that the activity of endogenous TGase and denaturation of muscle proteins have different optimal temperature.

Beef gels containing 0.3% MTG and incubated at 40–60C showed higher mechanical properties than control gels. Thus, adding MTG and allowing muscle proteins to denature at a temperature slightly lower than the optimal for denaturation at the onset, avoiding protein aggregation, allows inducing the cross-linking of adjacent proteins. These results could be useful to improve the mechanical properties of beef gels with lower amounts of MTG.

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