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Properties of Fish and Beef Restructured by MTG Derived from *Streptomyces mobaraensis* Grown in Media Based on Enzymatic Hydrolysates of Sorghum

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Abstract

Rodríguez Castillejos G., Ramírez de León J., Bustos Vázquez G., Castillo Ruíz O. (2017): Properties of fish and beef restructured by MTG derived from *Streptomyces mobaraensis* grown in media based on enzymatic hydrolysates of sorghum. Czech J. Food Sci., 35: 517–521.

The efficiency of microbial transglutaminase (MTG) obtained from *Streptoverticillum ladakanaum* fermentation of sorghum grain and DDGS hydrolysates (HMTG) in increasing the mechanical properties of restructured meat and fish products was evaluated in this study. Gels were obtained by adding HMTG or commercial MTG at 0.3 U/g, and controls lacked enzyme. All treatments were supplemented with 2.0% NaCl. The gels with enzyme showed a lower amount of expressible water, similar to those obtained with CMTG (6% for fish gels and 8% for beef gels). Texture values were also similar. The results showed the feasibility of employing MTG obtained from sorghum hydrolysates.

Keywords: microbial; sorghum; culture media; transglutaminase

The food industry is constantly evaluating new ways in which to improve food products. The meat industry, in particular, has succeeded in transforming low-cost cuts into attractive consumer foods. This has been achieved through the use of additives such as microbial transglutaminase (MTG), which allows the obtention of "restructured meat" (Castro-Briones *et al.* 2009a,b). Enzymes are widely used in food production due to their low or non-existent toxic potential. MTG is used as a cold-set binder in meat, to improve the mechanical properties of meat and dairy products, as well as in new plant-based products such as soybeans (Castro-Briones *et al.* 2009a; Guerra-Rodriguez & Vazquez 2013). Transglutaminases are able to modify the functional properties and textures of proteins by incorporating amines into the latter, creating products with greater consistency and better quality (Kieliszek & Misiewicz 2014). These enzymes are widely distributed in living organisms and can be found in mammal and fish tissues, plants and microorganisms (Griffin *et al.* 2002; Yu *et al.* 2008; Guerra-Rodriguez & Vázquez 2013); they have a variety of biological functions. Proteins are responsible for many of the properties of food and any change in them produces significant physicochemical changes. MTG introduces covalent bonds, which can be used to improve the functional properties of food, such as viscosity, solubility, elasticity, emulsifying capacity, water retention, formation of gels and foams and others (Guerra-Rodriguez & Vazquez 2014). The use of MTG in the food industry started with the production of surimi products in Japan (Jaros *et al.* 2006). MTG can restructure low-cost meat cuts from different species and enhance their value on the market for processed products (Lee & Park 2002). In the meat industry, mainly for beef and pork, meat scraps have reduced commercial value; therefore,

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several methods have been sought to restructure these cuts and improve their appearance. Previous restructuring methods involved the use of salt and thermal treatment (Huffman *et al.* 1981); the products thus restructured had to be frozen or pre-cooked to extend their shelf-life; plasma MTG was used to avoid this, although it has been an expensive process because of the difficulty in enzyme purification (Ando *et al.* 1989; Jaros 2006). Enzymes derived from microorganisms are very useful in the food industry. Therefore, the aim of this study was to evaluate the effectiveness of MTG isolated from media containing hydrolysates of red sorghum and DDGS. For this, we obtained gels of beef and fish and evaluated their colour, texture and water-holding capacity.

MATERIAL AND METHODS

Raw material. Fresh Lisa (*Mugil labrosus*) were eviscerated and filleted at facilities using a fish processor, thoroughly rinsed with cold tap water and stored on ice; beef was obtained from a butcher shop in the city of Reynosa, Tamaulipas; excess fat and connective tissue were removed. The MTG was obtained in our laboratory from culture medium based on glucose-rich syrup obtained by enzymatic hydrolysis of red sorghum grains supplemented with DDGS (Rodriguez-Castillejos *et al.* 2014). We used commercial MTG transglutaminase as a control. Four treatments were compared: one using 0.3 U/g MTG obtained from hydrolysates, another with 0.3 U/g of commercial MTG and two without enzyme. All treatments were supplemented with 2% NaCl.

Beef and fish gels. The restructured products were obtained using cutting, tumbling and mixing methods in the presence of salt. Beef and fish fillets were washed and drained before being weighed and chopped into pieces of approximately 5 cm; then, pieces were placed in a cutter (Hobart 84145; Hobart Inc., USA); 0.3% of enzyme and 2% of NaCl were added according to each treatment. The paste was stuffed using stainless steel tubes with a capacity of approximately 100 g and tubes were capped before immersion in water at 40°C (fish gels) or 50°C (beef gels) for 0.5 h followed by immersion in water at 90°C for 15 minutes. After cooking, all treatments were submitted to a thermal shock by immersion in cold water. Then, they were refrigerated and removed from their moulds. Finally the gels were then stored in plastic bags and refrigerated until used for further analysis.

Mechanical properties. Mechanical properties were determined using a TA-plus texture analyser (Lloyd Instruments, Australia). For compression, a cylindrical aluminium probe with a diameter of 50 mm was used. The samples were compressed to about 80% of their initial size using a compression rate of 60 mm/minutes. Sample values of maximum fracture velocity, strength and hardness were obtained using the NEXYGEN Plus data analysis software.

Colour determination. Colour attributes were determined using a HunterLab MiniScan XE Plus colorimeter (model 45/0-L; Hunter Associates Laboratory, USA). The samples were placed on a white background and three measurements were made to obtain the parameters of brightness (L^*), redness (a^*), and yellowness (b^*) on the Hunter scale. With these data, we calculated the parameters of chroma ($[a^*2 + b^*2]1/2$) and hue angle (arc tan b^*/a^*).

Determination of expressible water. To analyse expressible water for each treatment, 3 ± 0.2 g of sample were weighed and wrapped with Whatman paper No. 1, and then centrifuged at 1000 *g* for 15 min at 5°C. Subsequently, the samples were weighed and the extracted water percentage was calculated by dividing the final weight by the initial weight and multiplying the result by 100.

Statistical analysis. All experimental measurements were carried out in triplicate and the mean values are given. One-way analysis of variance (ANOVA) was conducted using Statgraphics-5 (STSC Inc., USA). Differences among means were analysed using the Tukey Test ($P < 0.05$).

RESULTS AND DISCUSSION

Colour attributes in restructured products. Table 1 shows the colour attributes of the fish and beef gels. For fish gels the a^* and b^* values were within the range of greens ($a-$) and yellows ($+b$). The H^* values (hue) were slightly higher than 90 (91.5–94.4), which identifies them as yellow-green gels, but the low value of chroma (C^*) (12.2–14.31) and the high L^* value (59.8–63.3) places them within the range of grey hues for most consumers. For beef gels, the a^* value for all samples was positive, indicating a reddish hue. The b^* in all gels was also positive, indicating yellowish hues. The L^* was close to 50 (48.7–50.6), indicating a slightly darker colour. The H^* range of 71.6–72.6 indicates a reddish-brown colouration, which has moderate intensity according to the C^*,

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Table 1. Colour attributes of gels

Treatment	L^*	a^*	b^*	C^*	H^*
Fish control (90°C/15 min)	61.54^{a} (± 2.48)	-0.4^{a} (± 0.10)	14.49^{a} (± 0.98)	14.49^{b} (± 0.98)	91.59^{a} (± 0.29)
Fish control (50°C/30 min + 90°C/15 min)	62.94^{a} (± 2.31)	-0.35^{a} (± 0.04)	14.37^{a,b} (± 1.21)	14.38^{b} (± 1.21)	94.41^{b} (± 0.24)
Fish CMTG (50°C/30 min + 90°C/15 min)	63.34^{a} (± 3.17)	-0.81^{a} (± 0.38)	12.20^{b} (± 0.68)	12.23^{a} (± 0.64)	93.86^{a,b} (± 2.03)
Fish HMTG (50°C/30 min + 90°C/15 min)	59.82^{a} (± 1.01)	-0.51^{a} (± 0.43)	13.38^{a} (± 0.10)	13.39^{a,b} (± 0.10)	92.2^{a} (± 1.82)
Beef control (90°C/15 min)	49.51^{a} (± 1.82)	5.36^{a} (± 0.10)	16.14^{a} (± 0.23)	17.01^{a} (± 0.25)	71.63^{a} (± 0.20)
Beef control (50°C/30 min + 90°C/15 min)	48.70^{a} (± 1.91)	5.45^{a} (± 0.21)	16.41^{a} (± 0.18)	17.30^{a} (± 0.18)	71.62^{a} (± 0.72)
Beef CMTG (50°C/30 min + 90°C/15 min)	49.02^{a} (± 1.35)	5.32^{a} (± 0.26)	16.26^{a} (± 0.27)	17.10^{a} (± 0.32)	72.64^{a} (± 0.65)
Beef HMTG (50°C/30 min + 90°C/15 min)	50.60^{a} (± 0.59)	5.25^{a} (± 0.16)	16.30^{a} (± 0.19)	17.12^{a} (± 0.22)	72.16^{a} (±0.35)^{a}

^{a,b}different letters indicate significant differences ($P \leq 0.05$); average values of five repetitions and standard deviation

which ranged from 17–17.3. There were no significant differences ($P \leq 0.05$) in the L^*, C^*, and h^* attributes between the gels treated with commercial enzyme and those treated with enzyme isolated from enzymatic hydrolysates. This result is important because the colour of food is a highly significant characteristic in consumer taste preferences. Tellez-Luis *et al.* (2004), using MTG obtained from *S. ladakanum* in hydrolysates of sorghum straw (SMTG) medium, reported that the L^* attribute decreased in samples containing commercial SMTG. Karayannakidis *et al.* (2008) reported that the addition of MTG (commercial) had a positive effect on the whiteness index of heat-induced sardine fish muscle protein; other studies reported that the addition of MTG could reduce the whiteness of gels from Indian mackerel fish protein isolates and suggested that the denser gel network was induced by the fact that MTG exhibits

Table 2. Texture analysis parameters of gels

Sample	Max work	Module	Max effort	Max deformation	Hardness 60%
Fish control (90°C/15 min)	0.034^{b} (± 0.004)	0.226^{b} (± 0.009)	0.057^{b} (± 0.002)	0.603^{b} (± 0.070)	4.056^{b} (± 0.186)
Fish control (50°C/30 min ± 90°C/15 min)	0.033^{b} (± 0.005)	0.248^{b} (± 0.013)	0.061^{b} (± 0.004)	0.454^{b} (± 0.045)	3.833^{b} (± 0.452)
Fish CMTG (50°C/30 min ± 90°C/15 min)	0.140^{a} (± 0.007)	0.215^{a,b} (± 0.011)	0.142^{a} (± 0.005)	0.986^{a} (± 0.023)	10.774^{a} (± 0.253)
Fish HMTG (50°C/30 min ± 90°C/15 min)	0.154^{a} (± 0.007)	0.206^{a} (± 0.009)	0.151^{a} (± 0.004)	1.021^{a} (± 0.024)	11.061^{a} (± 0.209)
Beef control (90°C/15 min)	0.022^{b,c} (± 0.005)	0.323^{b,c} (± 0.034)	0.062^{a} (± 0.007)	0.349^{b} (± 0.045)	3.756^{b} (± 0.695)
Beef control (50°C/30 min ± 90°C/15 min)	0.018^{c} (± 0.001)	0.284^{c} (± 0.018)	0.055^{a} (± 0.003)	0.321^{b} (± 0.010)	3.087^{b} (± 0.149)
Beef CMTG (50°C/30 min ± 90°C/15 min)	0.031^{a,b} (± 0.006)	0.200^{a,b} (± 0.096)	0.065^{a} (± 0.007)	0.472^{a} (± 0.054)	4.433^{a,b} (± 0.709)
Beef HMTG (50°C/30 min ± 90°C/15 min)	0.037^{a} (± 0.013)	0.210^{a} (± 0.058)	0.064^{a} (± 0.011)	0.572^{a} (± 0.135)	4.518^{a} (± 0.873)

$^{a–c}$different letters indicate significant differences ($P \leq 0.05$); average values of five repetitions and standard deviation

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Table 3. Percentage of expressible water of gels

Treatment	Expressible water (%)
Fish control (90°C/15 min)	10.44[b] (± 0.50)
Fish control (50°C/30 min ± 90°C/15 min)	10.04[b] (±0.61)
Fish CMTG (50°C/30 min ± 90°C/15 min)	6.37[a] (± 0.67)
Fish HMTG (50°C/30 min ± 90°C/15 min)	6.48[a] (± 0.73)
Beef Control (90°C/15 min)	12.7[b] (± 1.38)
Beef control (50°C/30 min ± 90°C/15 min)	11.93[b] (±0.71)
Beef CMTG (50°C/30 min ± 90°C/15 min)	8.17[a] (± 0.81)
Beef HMTG (50°C/30 min ± 90°C/15 min)	7.38[a] (± 1.27)

[a,b]different letters indicate significant differences ($P \leq 0.05$); average values of three repetitions and standard deviation

high light absorption, leading to a darker colour of gels (Chanarat & Benjakul 2013). Castro-Briones *et al.* (2009a) reported L^*, h^*, and C^* of 48.69, 69.35, and 13.56, respectively, in restructured beef gels; the h^* value indicates the gels had a brownish colour, but the low values of lightness and C^* indicate that the gels could be perceived as greyish by consumers.

Texture analysis of the restructured products. A texture analysis of meat and fish gels was performed using the method of uniaxial compression. The results showed no significant differences ($P \leq 0.05$) between the gels made with commercial enzyme and those made with enzyme isolated from media based on enzymatic hydrolysates. The gels with enzyme showed better texture properties than the controls (Table 2). However, there was no difference between the gels treated with CMTG and HMTG. The texture of food is a key parameter and is related to protein content and activity. MTG improves gel properties by inducing the formation of non-disulphide covalent bonds between the glutamine and lysine residues of proteins, which favours the formation of a stronger three-dimensional network (Hu *et al.* 2015). Gels treated with enzyme in the form of either HMTG or CMTG showed higher strength and lower deformability than those without MTG. Hu *et al.* (2015) evaluated the effects of curdlan and MTG on the gelling properties of hairtail muscle protein in the form of 0.4 U/g of meat paste; a marked increase in textural parameters was observed compared to samples without MTG. Monteiro *et al.* (2015) observed an increase in hardness in restructured tilapia steaks containing 0.5% MTG. The results obtained from the texture analysis indicated that crude extract containing HMTG could improve the mechanical properties of restructured fish and beef products; also, this increase could be attributed to residual casein, peptone and yeast extract from the culture medium (Téllez-Luis *et al.* 2004). The results of hardness were low in restructured beef compare with restructured fish, because beef muscle proteins are considered non-setting proteins, as they do not form gels at low temperatures (0–40°C); in fish proteins, the setting is caused by denaturation of myofibrillar proteins and the presence and activity of the endogenous transglutaminase. However, in beef proteins, this concordance does not exist. The use of MTG improves the mechanical properties of beef gels. Further, a pre-heating step improves enzyme efficiency as well as the mechanical properties of the beef gels (Castro-Briones *et al.* 2009a,b).

Expressible water and water holding capacity. The content of expressible water is inversely related to the water holding capacity (WHT); therefore, a low percentage of expressible water means a high percentage of WHT. The results showed significant differences ($P \leq 0.05$) between gels with MTG and controls (Table 3); enzyme improved WHT in gels. MTG promotes the formation of protein-protein interaction by forming a three-dimensional network that allows the entrapment of water. Téllez-Luis *et al.* (2004) reported no difference between a control without MTG and a treatment with SMTG in restructured fish; this was associated with a higher level of peptone and yeast extract in the SMTG. The HMTG used in the present study had a positive effect on the WHT parameter, confirming the activity of the enzyme. HU *et al.* (2015) observed that expressible water decreased significantly (12.94–6.43) when 0.4% MTG was added to hairtail muscle protein paste. Moreno *et al.* (2008) reported a value of 14.12 in a minced hake muscle gel using 10% of MGT.

CONCLUSIONS

The obtained results indicate that CMTG and HMTG increased the mechanical properties of restructured fish and beef. MTG obtained from enzymatic hydrolysates improved mechanical properties

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and water holding capacity, had a slight effect on colour attributes, and exhibited higher efficiency compared to CMTG. The high cost of commercial MTG makes the obtention of transglutaminase from enzymatic sorghum grain hydrolysates a viable alternative for the food industry.

References

Ando H., Adachi M., Umeda K., Matsuura A., Nonaka M., Uchio R., Motoki M. (1989): Purification and characteristics of a novel transglutaminase derived from microorganisms. Agricultural and Biological Chemistry, 53: 2613–2617.

Castro-Briones M., Calderón G.N., Velazquez G., Rubio M. S., Vázquez M., Ramírez J.A. (2009a): Mechanical and functional properties of beef products obtained using microbial transglutaminase with treatments of pre-heating followed by cold binding. Meat Science, 83: 229–238.

Castro-Briones M., Calderón G.N., Velazquez G., Salud-Rubio M., Vázquez M., Ramírez J.A. (2009b): Effect of setting conditions using microbial transglutaminase during obtention of beef gels. Journal of Food Process Engineering, 32: 221–234.

Chanarat S., Benjakul S. (2013): Effect of formaldehyde on protein cross-linking and gel forming ability of surimi from lizardfish induced by microbial transglutaminase. Food Hydrocolloids, 30: 704–711.

Griffin M., Casadio R., Bergamini C.M. (2002): Transglutaminases: nature's biological glues. Biochemical Journal, 368: 377–396.

Guerra-Rodríguez E., Vázquez M. (2013): Technical and economical evaluation of microbial transglutaminase production on enzymatic hydrolysates of potato (*Solanum tuberosum*). CyTA-Journal of Food, 11: 277–284.

Guerra-Rodríguez E., Vázquez M. (2014): Evaluation of a novel low-cost culture medium containing exclusively milk, potato and glycerol for microbial transglutaminase production by *Streptomyces mobaraensis*. Chemical Engineering Research and Design, 92: 784–791.

Hu Y., Liu W., Yuan C., Morioka K., Chen S., Liu D., Ye X. (2015): Enhancement of the gelation properties of hairtail (*Trichiurus haumela*) muscle protein with curdlan and transglutaminase. Food Chemistry, 176: 115–122.

Huffman D.L., Ly A.M., Cordray J.C. (1981): Effect of salt concentration on quality of restructured pork chops. Journal of Food Science, 46: 1563–1565.

Jaros D., Partschefeld C., Henle T., Rohm H. (2006): Transglutaminase in dairy products: chemistry, physics, applications. Journal of Texture Studies, 37: 113–155.

Karayannakidis P.D., Zotos A., Petridis D., Taylor K.A. (2008): Physicochemical changes of sardines (*Sardina pilchardus*) at –18°C and functional properties of kamaboko gels enhanced with Ca^{2+} ions and MTGase. Journal of Food Process Engineering, 31: 372–397.

Kieliszek M., Misiewicz A. (2014): Microbial transglutaminase and its application in the food industry. A review. Folia Microbiologica, 59: 241–250.

Lee E.Y., Park, J. (2002). Pressure inactivation kinetics of microbial transglutaminase from *Streptoverticillium mobaraense*. Journal of Food Science, 67: 1103–1107.

Monteiro M.L.G., Mársico E.T., Lázaro C.A., da Silva A.C.V.C., da Costa Lima B.R.C., da Cruz A.G., Conte-Júnior C.A. (2015): Effect of transglutaminase on quality characteristics of a value-added product tilapia wastes. Journal of Food Science and Technology, 52: 2598–2609.

Moreno H.M., Carballo J., Borderías A.J. (2008): Influence of alginate and microbial transglutaminase as binding ingredients on restructured fish muscle processed at low temperature. Journal of the Science of Food and Agriculture, 88: 1529–1536.

Rodríguez-Castillejos G.C., Tellez-Luis S.J., Vázquez M., Lois-Correa J.A., Ramírez J.A. (2014): Evaluation of sorghum grain hydrolysates and dried distillers grains with solubles for the production of microbial transglutaminase. CyTA-Journal of Food, 12: 115–120.

Téllez-Luis S.J., Ramírez J.A., Vázquez M. (2004): Application in restructured fish products of transglutaminase obtained by *Strepto verticillum ladakanaum* in media made from hydrolysates of sorghum straw. Journal of Food Science, 69: FMS1–FMS5.

Yu Y.J., Wu S.C., Chan H.H., Chen Y.C., Chen Z.Y., Yang M.T. (2008): Overproduction of soluble recombinant transglutaminase from *Streptomyces netropsis* in *Escherichia coli*. Applied Microbiology and Biotechnology, 81: 523–532.

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