PE4.26 Transglutaminase improves the textural and structural properties of chicken skeletal, smooth, and cardiac muscles 97.00

Abdulatef Ahhmed (01-feb) abdo772201@yahoo.co.uk, S Kawahara(1), M Muguruma 1,
(1)University of Miyazaki, Japan
(2)Gheran Higher Center for Agricultural Technologies, Japan

Abstract: **This study examined the effects of microbial transglutaminase (MTG; 3.1 mg/ml) on chicken skeletal, smooth, and cardiac muscles. Although the three muscle types were obtained from the same bird, the effects of MTG addition were not uniform. All the muscle types showed a significant increase in the breaking strength ($P < 0.01$), but skeletal muscle exhibited the maximum increase. All samples showed a decrease in the fluorescence intensity and a significant reduction in the concentration of proteins that were extracted in a high ionic strength solution ($P < 0.05$). Scanning electron microscopy images and histological studies revealed that different muscle types had different physical structures and frameworks after MTG treatment, which is a reflection of the differences in the reaction specificity of MTG with different muscle proteins. Cooking loss data suggested that MTG did not have any negative effect on water retention during cooking. Results suggest that MTG can function on all muscle types that are mechanically processed for different industrial applications. As a result, MTG aggregates muscle proteins in different ways that improves their organoleptic properties such as texture, appearance, and water retention.**

A. M. Ahhmed is with the *Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan,* phone: +81-98558-7203; fax: +81-98558-7203; (e-mail: abdo772201@yahoo.co.uk), on leave from Gheran Higher Centre for Agricultural Technologies,*P.O.B, 151-Tripoli, Libya, Phone +218-21-4870254; fax +218-21- 4870151*

S. Kawahara is with the *Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan,*(e-mail:a04206u@cc.miyazaki-u.ac.jp).

M. Muguruma is with the *Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan,*(e-mail: Muguruma@cc.miyazaki-u.ac.jp).

***Keywords: Chicken,* skeletal, smooth, and cardiac muscles; transglutaminase**

I. INTRODUCTION

Microbial Transglutaminase (MTG) induces proteins to adhere and form a strong network structure. Addition of MTG primarily leads to changes in proteins and the formation of protein structures, especially with the myosin heavy chain (MHC) [1]. MTG treatment changes the texture and gel strength of meat and meat proteins by forming a bond between Gln and Lys, which improves the rigidity and gel elasticity of meat products, thereby avoiding some undesirable attributes such as stickiness, high viscosity, and excessive adhesiveness [2]. MTG has muscle type-specific effects on meat proteins, and its reactions are complex, variable, and influenced by many factors. The three muscle types differ in many ways, including their function, location, shape, fiber direction, relative fiber size, origin of insertion, and protein composition. The objective of this study was to examine the effects of MTG on the textural, physicochemical, and structural properties of chicken skeletal, smooth, and cardiac muscles.

II. MATERIALS AND METHODS

2.1. Meat and preparation of samples

Japanese broiler chickens (8 weeks old) were sourced from a butcher in Miyazaki, Japan. After slaughter and upon arrival in the laboratory, the pH of the three muscles was determined and found to be 5.5. The meat was minced in a meat grinder at ~4°C and formed into a sausage by mixing 50 g ground meat, 30 ml distilled water, 1.4 g NaCl, and 0.21 g sodium pyrophosphate. Subsequently, 1 ml MTG solution (or 1 ml water in the control) was added, and the paste was extruded into a clear plastic casing (diameter = 25-mm). The positive and control groups were divided into two subgroups and subjected to different heat treatment processes. One subgroup was incubated at 40°C for 30 min, while the second was incubated at 78°C for 30 min to prepare well-cooked meat.

2.2. Preparation of the MTG solution

The enzyme was isolated by and the MTG powder was obtained from Ajinomoto Co., Japan and dissolved in 20 mM NaCl and 100 mM NaN_3 The MTG stock solution used in this study had a concentration of 3.1 mg/ml.

2.4. Testing the textural properties

The breaking strength of all samples was evaluated at room temperature using a knife fitted with a creep-meter (Rheoner II, Yamaden Co. Ltd., Tokyo, Japan), and the knife creep speed was set at 1 mm/s.

2.5. Protein extraction

The samples were dissolved in two different, the first consisted of 50 mM imidazole-HCl (pH 6.0) and 2 mM EDTA; this is a low ionic strength solution designed to extract water-soluble proteins (WSP). The

second contained 0.09 M KH_2PO_4, 0.06 M K_2HPO_4, 0.3 M KCl, and 1 mM ATP (pH 6.5); this is a high ionic strength solution that is also known as (GS-ATP). To extract the proteins, 28 ml of either the WSP or GS-ATP solution was added to 2 g sample (positive and / or control) and homogenized in a polytron homogenizer three times for 30 s at 10-s intervals. The mixtures were centrifuged at 12,000 rpm for 30 min at 4°C in a Himac CR 20E centrifuge. The supernatants were recovered and filtered through filter paper No. 5A, and the filtrate was used for the experiments.

2.6. Surface hydrophobicity

The surface hydrophobicity of the proteins before and after MTG addition was determined by the ANS method. A 50-ml aliquot of 8 mM ANS in 10 mM phosphate buffer (pH 7.0) was added to 4 ml protein solution (2.5 mg/ml for each muscle type) that had been extracted in GS-ATP buffer. The fluorescence intensity of the ANS-protein conjugate was measured in a spectrofluorimeter at an excitation wavelength of 365 nm and an emission wavelength of 470 nm.

2.7. Electrophoresis and evaluation of MHC band intensity

The molecular masses of the polymers produced by MTG treatment were determined by SDS-PAGE at ~20 mA/gel, and gradient slab gel (7.5%–20% (w/v) acrylamide) containing 2-mercaptoethanol. MHC band intensity was evaluated as described previously [3]. The band patterns were examined using an intensity meter.

2.8. Scanning electron microscopy (SEM)

Three gels of each specimen were fixed in 2% (v/v) glutaraldehyde, 0.13% (w/v) sodium phosphate buffer, and 1.33% (v/v) osmium tetroxide. These were dehydrated by immersion in a series of ethanol concentrations; and finally immersed in isoamyl acetate. The samples were stored in *t*-butyl alcohol (2-methyl-2 propanol). After dehydration, critical point drying was carried out the fragments were coated with platinum using an ion sputter instrument. All samples were photographed at a magnification of 15,000.

2.9. Histological images

Histological imaging was carried out essentially as described earlier [4] but with slight modifications. The muscle samples were immersed in 10% MTG at room temperature for 120 min, while the control samples were immersed in distilled water under the same conditions. The stained slices were mounted on a glass microscope slide and inspected and photographed under the 20× lens of a transmission electron microscope.

2.10. Cooking loss (CL)

The weight of the samples was checked before and after heat treatment. The weight measurements of control samples were carried out at room temperature. The CL was measured and expressed as a percentage of the raw material as follows:
$(S_2 - S_1)/S_1 \times 100$. Where S_1 is the weight before cooking, and S_2 is the weight after cooking.

III. RESULTS AND DISCUSSION

3.1. Changes in the breaking strength

Figures 1 and 2 show the values of the breaking strength of chicken skeletal, smooth, and cardiac muscles that were heated at 40°C and 78°C for 30 min, respectively. In comparison to the control samples, the values increased significantly in all treated samples ($P<0.01$), and this was associated with the presence of MTG. The breaking strength of the skeletal muscle sample after MTG treatment was much higher than that of the other two muscle types treated with the same amount of MTG. The breaking strength of MTG-treated samples of smooth and cardiac muscles did not differ significantly between the muscle types, but the values were significantly higher than those of the control samples. These results imply that MTG plays a functional role in the gel-forming process. The values of the breaking strength greatly differ between meat products, which originally based on differences in the muscle type.

3.2. Extractability of proteins

As a result, a few water-soluble proteins reacted with MTG, although we did not detect any bands in SDS-PAGE. Table 3 shows the protein concentration of GS-ATP solution extracts of skeletal, smooth, and cardiac muscles. The GS-ATP solution helps in the extraction of MHC. These data suggest that the decrease in protein extractability from all samples was significantly affected ($P<0.05$) by MTG addition. The amount of protein extracted in the GS-ATP solution from skeletal muscles was higher than that extracted from smooth and cardiac muscles. This suggests that although muscles can react with MTG, they do so in different ways that reflect the protein type present in each muscle.

3.3. Changes in surface hydrophobicity

Fluorescence intensity measurements indicated that the surface hydrophobicity of the proteins extracted from all muscle types decreased considerably as a result of MTG treatment (Fig. 3). The fluorescence intensities of the control samples were higher than those of the treated samples at all Gu-HCl concentrations and for each muscle type. Such a decrease in the surface hydrophobicity of highly hydrophilic proteins generally (treated samples) indicates that the polarity of amino acids has been

reduced. This also suggests that a considerable number of amino acids have interacted with each other as a result of MTG action. As more structures are created by MTG action, the hydrophobicity reduction is greater, and the physical properties also change to a greater extent. The higher the Gu-HCl concentration, the greater is the increase in fluorescence intensity. Moreover, the differences in the control samples probably arose from the entrapment of the amino acids inside protein strands or from the mechanical function of each muscle since there appeared to be fewer differences across different muscle types.

3.4. SDS-PAGE analysis

The pattern shows the effect of MTG on MHC bands in samples extracted in a GS-ATP solution (Fig. 5). Analysis of the extracted protein showed a variation in the density of the MHC bands together with some variation in the reaction ratio. The intensity (band area per mm^2) of the bands of skeletal, smooth, and cardiac muscles after MTG treatment was significantly reduced ($P < 0.05$). The original area of the MHC bands of all treated muscles was altered after MTG addition. It indicates that MTG acted upon MHC, resulting in a protein-protein network that improved the texture. It also suggests that the MTG action was not uniform among the three muscle types. In comparison to the control values, those for the skeletal, smooth, and cardiac muscle were 40%, 43%, and 47.3% lower, respectively.

3.5. SEM

Images of the control and MTG-treated samples of the skeletal, smooth and cardiac muscles are shown in figures 5-I, II &III, respectively. In the control skeletal samples, the structure is rough and the background has giant fibrous components and huge gaps. However, after MTG treatment, the background is more regular and the big gaps are closed and minimized. Images of control samples of smooth muscles also showed a coarse background and many peaks of small cuts of meat. Yet, after MTG addition, the background was also smooth and well ordered. Similarly, the background image of the control cardiac muscles was rough and unstructured but became ordered and smooth after MTG addition. Images of the samples of cardiac muscles after MTG treatment were the best and in these, the samples were neat. Results suggest that MTG greatly improved the textural properties as well as the appearance of the meat product. The data also suggests that the extent of reaction with MTG is muscle protein-dependent. In short, MTG can react with meat proteins and improve the texture, but the improvement is variable and depends on many factors, including differences in the morphology, mechanical and biological properties.

3.6. Changes in muscle histology

Clearly, there were many cleavages within the fibers and between the connective tissue, and these fissures (indicated by arrows) developed when the three samples were marinated in the MTG solution. The histological findings suggest that the level of alteration of muscle proteins depends on the MTG function. Differences were observed in the gross structure of the three types of muscle before and after MTG treatment. These differences provide evidence that different meat proteins are susceptible to MTG to different extents, but in all cases, the reaction occurs through the formation of a bond between Gln and Lys residues on the surface of muscle proteins. Therefore, we conclude that the reactions between MTG and meat proteins are both exogenous and endogenous.

3.7. Changes in CL

Skeletal muscles retained (maintained) much more water than cardiac and smooth muscles. The skeletal muscles and control samples of smooth muscles were unaffected by the low-heat treatment, however, the CL at 78°C increased slightly in both samples. This was in contrast to the cardiac muscles in which the CL for treated samples was slightly less than that of the control samples. Samples subjected to high-heat treatment were affected, and the CL was higher than that of the low-heat-treated samples. In comparison with the control samples, MTG-treated samples of skeletal muscles retained more water than MTG-treated smooth muscle samples (table 1). The CL did not differ significantly between the control and MTG-treated cardiac muscles. The three types of muscle varied in the amount of CL, which may be due to physiological, biochemical, and functional differences. As a result MTG did not have any negative effect on water retention during cooking.

IV CONCLUSION

The data show that the ability of MTG to catalyze the crosslinking of muscle proteins depends on the muscle type. As a result, we hypothesize that the variation in the final product is due to differences in the availability of glutamine and lysine in different muscle proteins. Protein specificity may be based on factors such as muscle type (smooth, skeletal, or cardiac), muscle function, and morphology. The results of this study suggest that the final products of MTG-treated chicken skeletal, smooth, and cardiac muscles differ considerably from those of the control samples. In general, the evidence suggests that MTG can function with all muscle types and improve the texture of meat products and/or maintain water retention.

REFERENCES

[1] Kawahara, S., Ahhmed, M. A., Ohta, K., Nakade, K., & Muguruma, M. (2007). Inconsistency in the improvements of gel strength in chicken and pork sausages include by microbial

transglutaminase. Asian-Australasian Journal of Animal Science, *20*, 1285-1291.
[2] Ahhmed, M. A., Kuroda, R., Kawahara, S., Ohta, K., Nakade, K., Aoki, T., & Muguruma, M. (2009). Dependence of microbial transglutaminase on meat type in myofibrillar proteins cross-linking. Food Chemistry, 112, 354-361.
[3] Astruc, T., Labas, R., Vendeuvre, L. J., Martin, L, J., & Taylor, G. R. (2008). Beef sausages structure sffected by sodium chloride and potassium lactate. Meat Science, 80, 1092-1099.

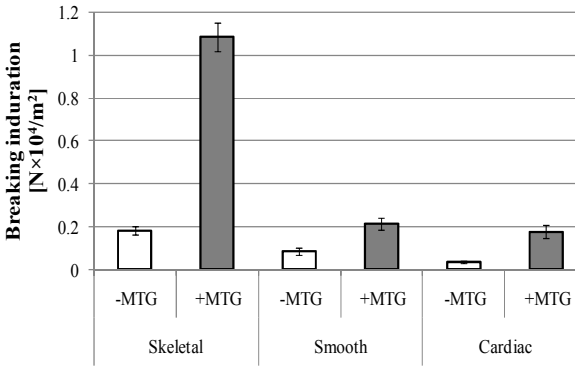


Fig. 1. Changes in breaking strength of the gel in chicken skeletal, smooth and cardiac muscles as a function of MTG and temperature. The samples were incubated at 40°C for 30 min. Bars with different superscripts are significantly different at (P <0.01).

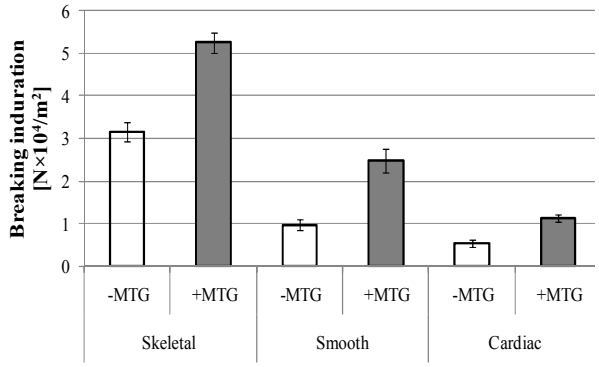


Fig. 2. Changes in breaking strength of the gel in chicken skeletal, smooth and cardiac muscles as a function of MTG and temperature.The samples were incubated at 78°C for 30 min. Bars with different superscripts are significantly different at (P <0.01).

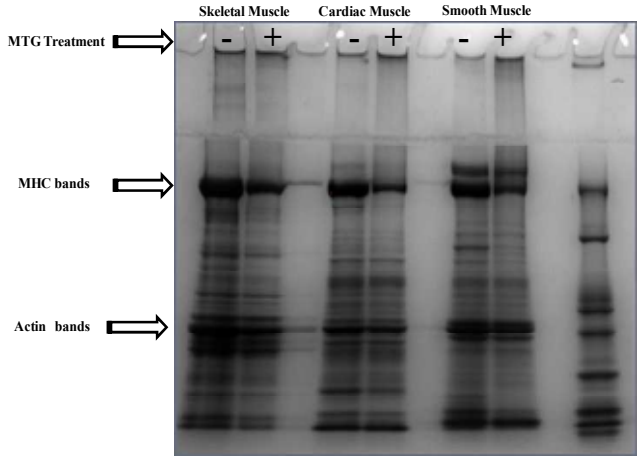


Fig. 4. The SDS-PAGE pattern illustrates how MTG influences MHC and other bands with low molecular mass in chicken skeletal, smooth and cardiac muscles. The samples were incubated at 40°C for 30 min. A huge reduction in MHC bands was observed.

Table 1. Changes in cooking loss of chicken skeletal, smooth and cardiac muscles associated with treatment of MTG.

Muscle type	MTG treatment	Percentage of cooking loss of samples cooked at 78°C (%)	
		Means	SEM
Skeletal	-	6.836 a	0.404
	+	9.9 ab	2.256
Smooth	-	37.19 c	1.424
	+	35.76 c	1.670
Cardiac	-	13.45 b	3.753
	+	13.36 b	1.253

Values within the same column with different superscripts are significantly different (P <0.05).

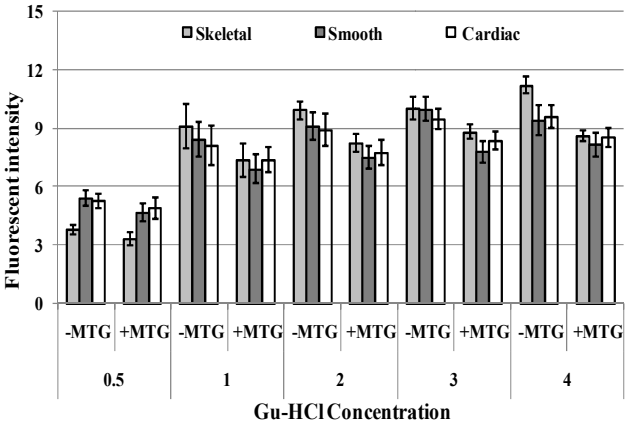
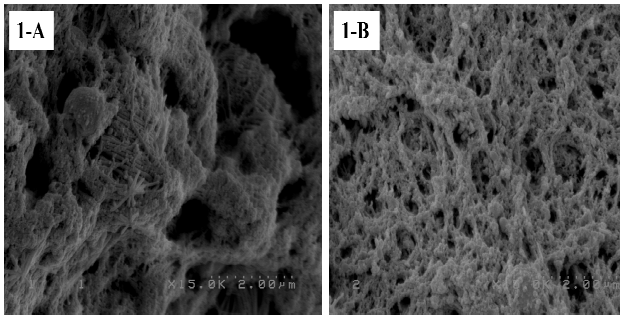


Fig. 3. The effect of Gu-HCl on the intensity of fluorescence (surface hydrophobicity) of crosslinked proteins and native proteins (both were extracted in GS-ATP solution). The samples were incubated at 40°C for 30 min.



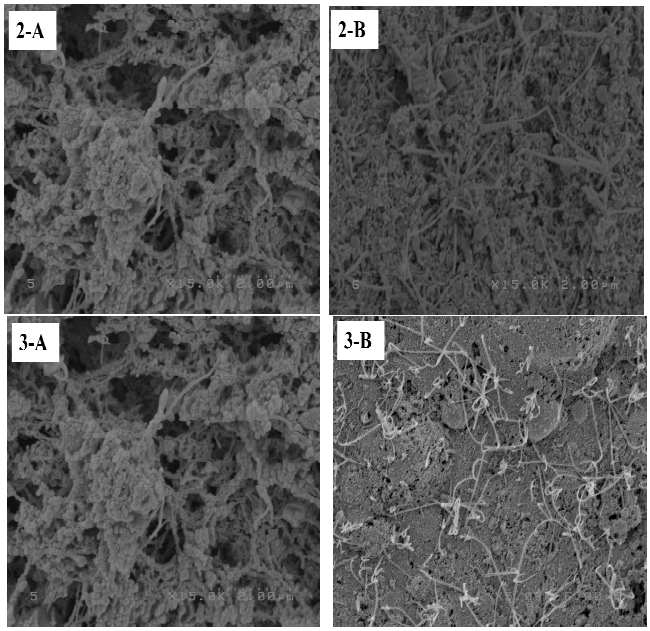


Fig. 5. Scanning electron microscope images of chicken skeletal (1), smooth (2) and cardiac (3) muscles. The samples were incubated at 40°C for 30 min. (A) Control samples before the addition of MTG; and (B) samples after the addition of MTG. The images were obtained at a magnification of 15,000×.