Asian-Aust. J. Anim. Sci.
Vol. 20, No. 8 : 1285 - 1291
August 2007

www.ajas.info

Inconsistency in the Improvements of Gel Strength in Chicken and Pork Sausages Induced by Microbial Transglutaminase

S. Kawahara, A. M. Ahhmed, K. Ohta, K. Nakade[1] and M. Muguruma*
Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan

ABSTRACT : This research investigated variation in the improvement of the texture of chicken and pork sausages induced by microbial transglutaminase (MTG). The extractability of myofibrillar proteins from these sausages as well as the ε-(γ-glutamyl)lysine (G-L) content were also investigated. MTG treatment of sausages significantly increased the breaking strength values in both meat types, especially for samples incubated at 40°C. However, values of the breaking strength in both meat types were increased differently. The variation in protein extractability of samples incubated at 40°C for both meat types could lead to some consideration of the mechanisms and the high accessions of myosin heavy chain (MHC) to MTG. SDS-PAGE analysis showed significant changes in the density of the bands after adding MTG, especially for the pork samples in which the bands disappeared totally. The G-L content in the presence of MTG was double that in control samples of both meat types. This study suggests that the binding ability of myofibrillar proteins with MTG is strong. This leads us to suggest that MTG functions positively with different improvements in the texture of chicken and pork products that are treated mechanically, such as sausages. Variability in gel improvement level between chicken and pork sausages was observed; this resulted from the variation in meat proteins in response to MTG, as well as to the original glutamyl and lysine content. (**Key Words :** Texture, Chicken, Pork, Microbial Transglutaminase, Gel Improvement, Protein Crosslinking)

INTRODUCTION

Second attribute after meat color that affects on the consumer decision at the point of purchase meat products is meat texture. Generally, meat texture perceive by many basic factors such as animals age, slaughtering way, carcass temperate at handling process, meat aging and mechanical process of the final product.

The chemical and physical properties of muscle tissue and the associated connective tissue are of utmost importance when considering the usefulness of meat as a food. Many processed meat products have been generated worldwide, such as sausages. Meat softening or stickiness (i.e. meat pasting) underlie to each circle link of many problem's chains in meat industry. An initial process is needed for making sausages, which is usually grinding the meat; this is creating some worries for meat industry.

The use of new material (bio-derived additives) sometimes can help to meet the challenges faced in meat manufacturing (Ahhmed et al., 2006). Using MTG in meat manufacture can help to avoid this kind of problem. Effectual functions of MTG are primarily involved with the protein compounds and reformations, especially with MHC. This enzyme and its usages in food and meat products in order to improve the properties of meat proteins has been investigated by many scientists in most world regions especially in Japan. Muguruma et al. (2003) showed that MTG can be used to improve the texture of chicken sausages even at reduced levels of phosphate. Katayama et al. (2006) in another study reported that MTG improves the properties of meat proteins and the texture of sausages manufactured from low-quality pork loin. MTG reticulates the proteins by transferring and combining amino groups of some proteins to other proteins. This process has unique characteristic, includes strengthening the texture of meat tissues after any mechanical process (i.e. grinding). Covalent attachment of essential amino acids of food proteins is also possible by TG reaction (Matheis and Whitaker, 1987). MTG has been known to catalyze the in-vitro crosslinking of a number of proteins, such as binding and textural properties of beef gel processed with MTG, κ-carrageenan, and egg albumin (Pietrasik, 2003). Further studies have been carried out on MTG with other proteins

* Corresponding Author: Michio Muguruma. Tel: +81-985-58-7203, Fax: +81-985-58-7203, E-mail: muguruma@cc.miyazaki-u.ac.jp
[1] Central Research Institute, Ito Ham Food Inc., Tokyo, Japan.
Received December 7, 2006; Accepted March 19, 2007

gelation of food proteins using MTG (Yokoyama et al., 2003).

The share force is highly related to the toughness force that resulted from protein-protein complexity, the interaction of proteins after adding MTG. One of the most important and significant indicators to clarify the influence of adding MTG is protein content as well as its extractability. Specifically, the low and high protein content and its extractability in sausages treated with MTG indicate whether MTG was interfered with some proteins. Extractability of MHC is considered to be a good index of the influence of adding MTG, simply because the proteins that responded to the MTG reaction are glutamyl and lysine.

SDS-PAGE demonstrated to show the new protein formations as a result of protein migration. However, it has also been used to measure the protein size after adding any additives that can change the interlocking protein structure. Erwanto et al. (2003) used SDS-PAGE to confirm modifications which developed after the addition of MTG on the gel properties of porcine collagen as well as to illustrate the intermolecular covalent crosslinking in gel porcine collagen. The ε-(γ-glutamyl)lysine (G-L) content of meats is used as an indicator to show the interactions in meat proteins that induced by MTG. In the present study, HPLC technique is used to show the content of ε-(γ-glutamyl)lysine. The gelation resulting from protein aggregation in foods, particularly meat products, is highly related to the enzyme's reactions as well as the biological activities of some additives.

To date, no study has been engaged to clarify the fundamental factors that institute the variation in the texture improvement between chicken and pork products that treated by MTG after mechanical process. Some meat products' makers are still querying about the use of MTG quantity with different meats, and they brought up many questions whether to use the same quantity of MTG to all types of meat or the additions of MTG must be at different level depending on meat type. Therefore, this study aimed to clear up the variation in the gel improvement of sausage texture of chicken and pork induced using MTG.

MATERIALS AND METHODS

Preparation of microbial transglutaminase solution

MTG was purified and produced from a variant of *Streptoverticillium mobaraense*, (Ando et al., 1989). The MTG used in the present study was obtained from Ajinomoto Co., Japan. MTG was dissolved in 20 mM NaCl, as described in a previous publication (Erwanto et al., 2005). The concentration was measured at 540 nm by the biuret method; the concentration of MTG in this study was found to be 3.72 mg/ml.

Preparation of meat and sausages

The cuts of chicken were thighs of broiler, slaughtered at age 8 weeks in a local chicken-slaughtering house then vacuum packaged and stored for 1 day in a chilled refrigerator. The pH of thighs was found to be 5.5. The cuts of pork meat used in this study were *semimembranosus* and *adductor* muscle from Japanese pigs; the age of the animals was around 6 months. The animals were slaughtered at Minami Kyushu Chikusan Kogyo Ltd., Kagoshima, Japan. The meat was vacuum-packaged and stored for 4 days in a chilled refrigerator after slaughtering and before being used in this study, the grade was defined as "fine" and the pH of the meat was found to be 5.6. Fatty tissues were removed from all the skeletal muscle blocks. The meat was minced in a meat grinder (MK-GL 20-W National), and the temperature was maintained at around 4°C. The sausages were prepared by mixing 50 g of the ground meat, 30 ml distilled water, 1.4 g of NaCl, and 0.21 g of sodium pyrophosphate, and then 1 ml of MTG was added. The proportion of MTG to MHC was estimated to be approximately 1:500. Puolanne and Terrell (1983) reported that salt increases the solubility of the muscle proteins and their water-binding ability. The purpose of adding NaCl was to increase the ability of the meat to hold water during cooking. On the other hand, sodium pyrophosphate was used to reverse the formation of the actomyosin complex, returning actin and myosin to their separate pre-rigor forms. All the additives and the minced meat were mixed in a meat blender (MK-K45 National, Japan); then the paste was placed in a funnel-shaped bag and extruded into a clear plastic casing. The paste was then stuffed into the casings; the diameter of the sausages is 25 mm. The sausages were divided into two groups: one group with transglutaminase (+MTG) and the other without transglutaminase (-MTG); sausages without MTG were considered to be the control samples among each group. Furthermore, each of these groups was divided into two groups based on the heat treatment. The first group was incubated at 40°C for 30 min, using a thermo-minder (Sm-05, Taitec, Tokyo, Japan); and the second group was cooked at 80°C for 30 min, using a water bath shaker (Personal-11 Taitec, Tokyo, Japan).

Measurement of breaking strength

The purpose of measuring the breaking strength was to observe the texture improvement of sausage as a function of MTG. The measurements were repeated six times for each experiment; the average, the means, and the standard deviation were calculated for the best ten values. The samples were prepared to have a height of 10-15 mm; primarily, the shape of the samples was 1-cm cubes. The measurements of breaking strength of the sausage samples were carried out using a knife fitted on a creep meter at room temperature. Each sample was set on the detachable

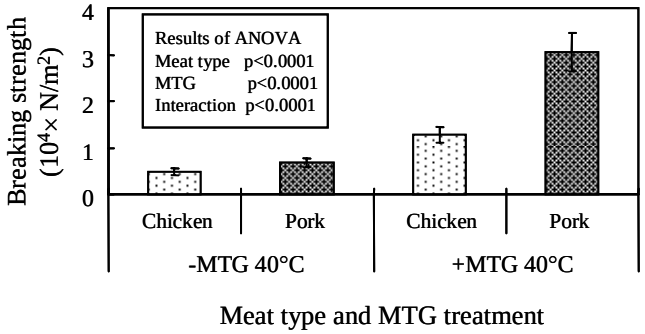

Figure 1. Changes in breaking strength of chicken and pork sausages as affected by the functions of MTG and heat treatment. The sausages cooked at 80°C for 30 min.

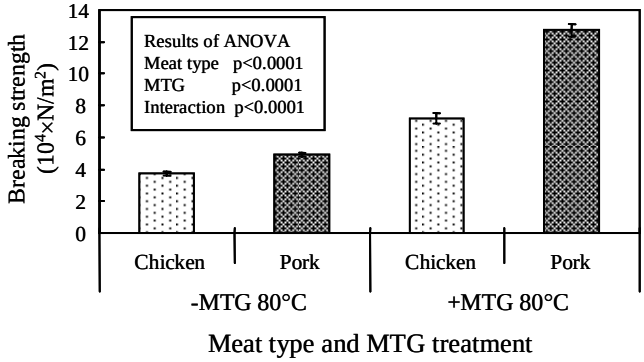

Figure 2. Changes in breaking strength of chicken and pork sausages as affected by the functions of MTG and temperature. The sausages incubated at 40°C for 30 min.

table of the rheometer (Yamaden Co. Ltd., Tokyo, Japan). Speed of the creep meter was adjusted and set at 1 mm/s as reported in a previous study (Muguruma et al., 2006).

Extraction of proteins

The samples were dissolved into two different solutions. The first was a low-ionic-strength solution, which was considered as water-soluble proteins (WSP) (50 mM imidazole-HCl (pH 6.0), 2 mM EDTA). The second was a high-ionic-strength solution, which was defined as Guba-Straub–adenosine triphosphate solution (GS–ATP) (0.09 M KH_2PO_4, 0.06 M K_2HPO_4, 0.3 M KCl, 1 mM ATP (pH 6.5)). The process of extracting the proteins was completed by separately adding 28 ml of each solution to 2 g sample of the sausage that had been divided into two groups according to the heat treatment as mentioned previously. The solutions along with the sausage samples were homogenized three times for 30 s, at 10 s intervals, by polytron homogenizer (Kinematica Co., Littau, Switzerland) at setting 4, the fourth fastest speed. Thereafter, the mixtures were centrifuged at 12,000 rpm for 30 min at 4°C in a Himac CR 20E centrifuge (Hitachi, Tokyo, Japan). Eventually the supernatants were taken out and filtered with filter paper No. 5A (Advantec Toyo K. Ltd., Tokyo, Japan); thus the final solution was used as an extracted protein solution. The protein concentration of the extracted protein solution was determined using the biuret method (Gornall et al., 1949) with bovine serum albumin used as a standard.

Preparation of SDS–PAGE

SDS–PAGE was used as a separation technique to diversify and disassociate the proteins according to their size after extracting them in different ionic strength solutions. It was carried out on gradient slab gel (7.5-17.5% acrylamide) with 2-mercaptoethanol at 20 mA/gel, employing the discontinuous buffer system of the method of (Laemmli, 1970).

Determination of ε-(γ-glutamyl) lysine content

The determination of ε-(γ-glutamyl)lysine content was carried out according to the method described by Kumazawa et al. (1993).

Statistical analysis

All the data in the present study represented at least five independent experiments and was expressed as the mean ±SEM. Two factorial analyses about meat type (chicken and pork) and MTG treatment (absence and presence of MTG) was carried out by means of 2-way ANOVA and followed by the Tukey method.

RESULTAS AND DISCUSSION

Changes in breaking strength

The gel improvement of sausages is very important from the consumers' point of view; therefore the changes in breaking strength of the sausages were determined to evaluate the effects of the addition of MTG on the texture. The breaking strength measurements were implemented in this research by a shear force instrument, which is designated by the resistance force of meats to being pressed by a cutter (knife). Otherwise, the sausages were subjected to the puncture test, which was measured using a knife, as mentioned previously. The sausages in the present study were divided into two groups as a function of the heat treatment. The first group was treated at 40°C for 30 min and the other group was cooked at 80°C for 30 min. Sakamoto et al. (1995) reported that in several food protein gels, breaking strength was influenced by incubation time or temperature. A particularly interesting approach in this study was proposed by preparing well-cooked sausages, with a cooking temperature of 80°C. In addition, the justification for using 40°C in our experiment was to investigate the reaction of MTG with MHC before the proteins become denaturized. Furthermore, we wanted to clarify the behavior of the proteins that engaged in the

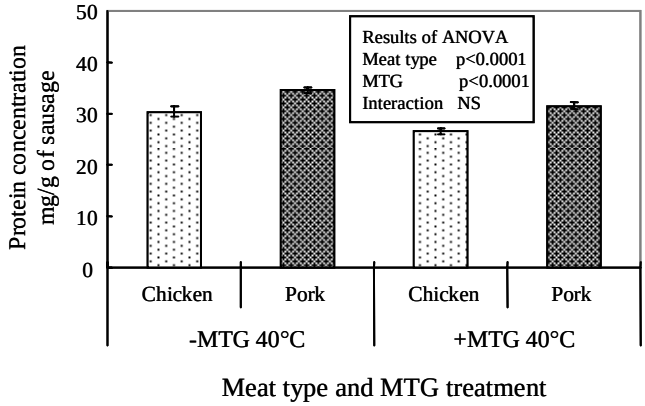

Figure 3. Protein concentrations by mg/g of chicken and pork sausages as affected by MTG. Proteins extracted in WSP solution (50 mM Imidazole-HCl (1 M pH 6.0), 2 mM EDTA. The sausages were incubated at 40°C for 30 min.

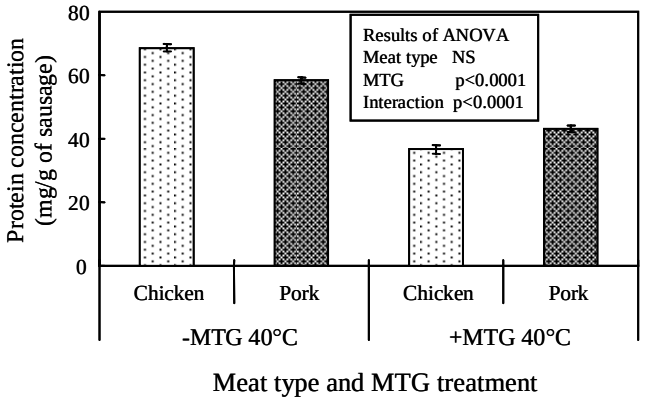

Figure 4. Protein concentrations by mg/g of chicken and pork sausages as affected by MTG. Proteins extracted in GS-ATP solution (0.09 M KH_2PO_4, 0.06 M K_2HPO_4, 0.3 M KCl, 1 mM ATP). The sausages were incubated at 40°C for 30 min.

interactions with MTG. As a result, the breaking strength values increased, which might be associated to the addition of MTG with the rise in temperature.

Figure 1 shows the values of breaking strength of chicken and pork sausages that treated at 40°C for 30 min. Figure 2 presents the values of the breaking strength of chicken and pork sausages treated at 80°C for 30 min. Muguruma et al. (1999) reported that the texture of chicken sausage was improved by the formation of ε-(γ-glutamyl)lysine crosslinks by the addition of MTG. As a result, the MTG's functional properties were implicated. Using MTG significantly increased the value of breaking strength in sausage samples ($p<0.0001$). However, MTG significantly and differently increased breaking strength values between meat types. MTG more affectively increased the breaking strength values of pork sausages even though those sausages cooked at 80°C. This leads us to suggest that the functional properties of MTG make it a very good additive to improve the texture of meat products such as sausages. However, the improvement was different between chicken and pork.

Protein extractability of chicken and pork sausages

Extractability of the protein in chicken and pork sausages in a water-soluble protein solution and Guba-Straub–ATP was investigated. The WSP solution was used to indicate whether the crosslinking of sausages that occurred with the MTG addition involved water soluble proteins or proteins elsewhere. A particular interest focused on using the GS-ATP solution. The extractability of myosin from the actomyosin complex and from crosslinked proteins induced by MTG is rare. Otherwise, the extractability of myosin from the actomyosin complex is high in the presence of ATP, such as extracting proteins in the GS-ATP solution. However, this is unachievable with the crosslinked proteins induced by MTG even though it extracted in GS-ATP solution for the possible reason that MTG reacts with MHC. GS-ATP solution can extract more myosin than the GS-solution, because of the dissociation of the rigor complex of actin and myosin by the addition of ATP (Muguruma et al., 1987).

In this part of the present study the sausages were incubated at 40°C for 30 min after the addition of MTG. The samples not treated with MTG served as control samples among each type. Protein concentration of the extractions from MTG-treated samples was decreased in both solutions. Values of samples which were extracted in WSP indicate that the protein concentration in the chicken samples was significantly lower than that in pork samples (Figure 3). However, among each type meat, the protein concentration of the samples treated by MTG was significantly less than the protein concentration of control samples. Samples treated by MTG gave lower concentrations than those considered as control samples. This leads us to suggest that some WSP reacted with MTG, although we did not detect any special bands in SDS-PAGE analysis of WSP solutions (data not shown).

Figure 4 provides protein concentrations in chicken and pork sausages extracted in GS-ATP solution. GS-ATP helps to extract MHC, and KCl was used as a salt to increase the ionic strength of the GS-ATP solution. These data revealed that the decrease in the protein extractability from the chicken and pork samples was significantly affected by MTG addition ($p<0.0001$). However, there was no significant difference among the meat type. These data suggest that MTG would principally crosslink proteins that dissolved in GS-ATP solution such as myosin.

SDS-PAGE pattern

In the SDS-PAGE analysis of the proteins of chicken and pork sausage treated with MTG, the proteins were extracted in a GS-ATP solution. SDS-PAGE was used to

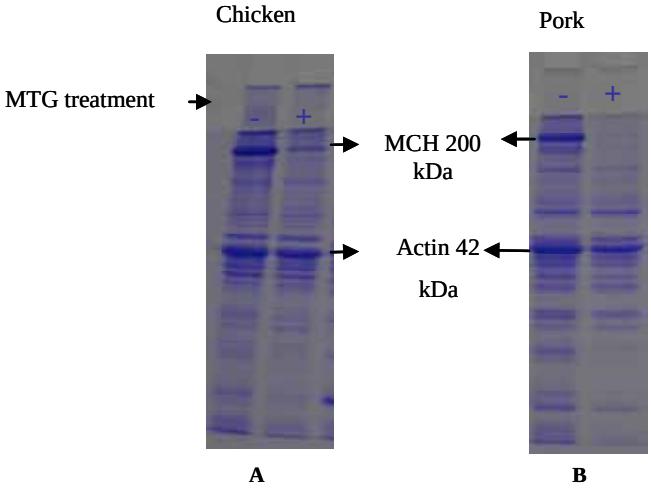

Figure 5. Changes in SDS-page pattern of chicken (A) and pork (B) sausage proteins. The pattern shows the MTG influence on MHC proteins and their bands intensity, which extracted by dissolving the sausage samples in GS-ATP solution. The sausages were incubated at 40°C for 30 min.

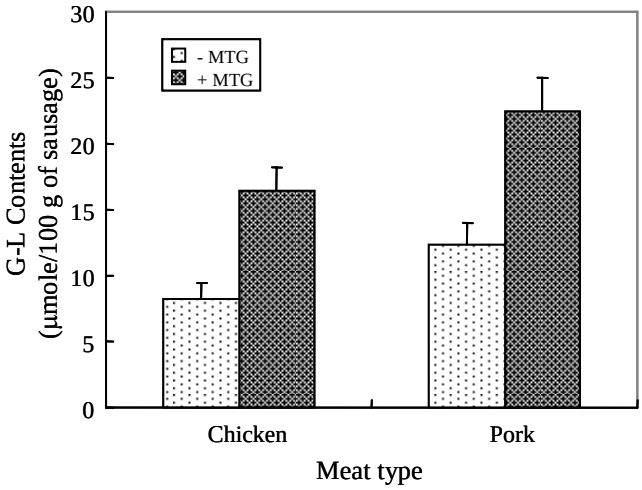

Figure 6. Changes in ε-(γ-glutamyl) lysine content (μmole/100 g of chicken (A) and pork (B) sausage. The sausage samples were incubated at 40°C for 30 min and then subjected to HPLC method.

examine the interaction between the myosin heavy chain molecules with MTG. Generally the proteins denature near to the cooking temperatures; therefore the bands of MHC in the samples incubated at 40°C are visible on SDS-PAGE gels. The SDS-PAGE pattern shows the appearance and disappearance of the MHC bands before and after adding MTG, respectively (Figure 5). Samples used in this part of the present study were samples of sausages treated at 40°C for 30 min and dissolved in a GS-ATP solution.

The analysis of extracted protein solutions illustrated the density variation in the bands of MHC; clearly showing the affect it had on MHC level in the absence and presence of MTG. However, it indicated how the bands were significantly reduced as a result of the addition of MTG. Decrease in the myosin heavy chain content on SDS-PAGE may have been due to crosslinking of the myosin heavy chain (Kumazawa et al., 1993). The label of the MHC in chicken control samples was quite large, quite dissimilar to those samples treated with MTG. Otherwise, the second lane clearly showed a significant decrease in the density of the MHC band. This reveals that the functional effects of MTG were on myosin heavy chain proteins and not elsewhere. Results from pork samples in this pattern did support our hypothesis that MTG reacts with MHC and not elsewhere. Furthermore, the pork samples prove that MTG had a significant effect on MHC, and the crosslinking was observed. Jiang et al. (2004) used SDS-PAGE to observe crosslinks of myosin heavy chain and low molecular mass compounds and the formation of ε-(γ-glutamyl)lysine in mackerel and hairtail.

Tseng et al. (2000) reported that the formation of isopeptide bonds is irreversible and contributes to strong protein–protein interactions that stabilize the network. Collectively, this suggests that the MHC proteins are the sovereign proteins that made a strong binding ability between MTG with myofibril protein. The results of SDS-PAGE analysis in the present study are in agreement with the findings of (Tsukamasa et al., 1993). Moreover, myosin is essential for the textured properties of meat products (Nonaka et al., 1989). In conclusion, the pattern of SDS-PAGE showed significant changes in the density of the bands, the band configuration is changed and that was clear especially for pork samples. Additionally, the discoloration was observed and it is unequivocal in MHC bands. In other words, MTG was a good protein-binding agent and could be a good additive to help the functionality of proteins, in order to improve the texture of meats, with marked diversity in the gel improvement level. It must, however, be recognized that there are other influential proteins; and similarly they may react as the influencing factors of the crosslinking battlefield, such as actin and collagen.

ε-(γ-glutamyl)lysine GL content analysis

The ε-(γ-glutamyl)lysine G-L content of freeze-dried samples was analyzed by HPLC after proteolytic digestion. Originally the samples were prepared from sausages treated at 40°C for 30 min. The G-L content values presented in Figure 6 clearly illustrate the amount of the proteins that engaged in crosslinks induced by MTG addition. The G-L content of chicken samples in the presence of MTG was increased twice as much as in control samples. A similar phenomenon was observed for the pork sample, which the values increased by a rate of 85% to control samples. The increment of G-L in pork samples treated with MTG roughly was double the amount in the control samples. This leads us to understand how the reaction was different between these types of meat sausages, even when these products had been treated using the same methods, such as

the addition of MTG, as well as when incubation time and temperature were held constant.

So, the content of G-L of sausages after adding MTG was significantly increased and the values of chicken samples entirely were lower than those of the pork samples. Sakamoto et al. (1995) suggested that MTG added to surimi would generate G-L crosslinks. Nio et al. (1986) mentioned that the addition of TG led to firmer gel networks through the formation of intermolecular G-L crosslinks. TG-induced gelation gave rise to more regular structure than seen in thermally induced gels (Chanyongvorakul et al., 1995). Nonaka et al. (1989) reported that microbial transglutaminase catalyzes the crosslinking between myosin heavy chains at low temperatures. In the present study, some antithetical data was obtained. In other words, the increase in G-L content was likely to occur during cooking, since it is highly probable that MTG catalyzed the reaction. It is likely that the G-L crosslinks could be formed through the MTG catalysis during the preparative process of surimi and/or surimi paste (Sakamoto et al., 1995). As a result, we suggest that the gel improvement diversity comes from the content of G-L, but we do not know yet whether the original amounts of glutamyl and lysine are higher in pork than in chicken; which might have resulted the variation in G-L content. However, pork treated samples showed values better than chicken treated samples (Ahhmed et al., 2005). The difference in G-L content in the absence and presence of MTG is due to the glutamine residues reacting at different rates depending on their location in the protein. This probably also resulted from the size and shape of the muscle. Certainly MTG has an influence on the meat proteins, especially meat products that are treated mechanically, such as by grinding or mashing. The addition of TG therefore appears to be a useful method to enhance the stability and yield of meat paste (Ruiz and Regenstein, 2002). Dimitrakopoulou et al. (2005) have conducted a study about the effects of salt and transglutaminase level and the processing conditions on quality characteristics of restructured pork shoulder, they showed that the consistency of the product was also significantly affected by the interaction between the transglutaminase level and the processing conditions as well as the interaction between the three main effects. Ikura et al. (1981) when they were examining the feasibility of using the TG reaction for fortifying food proteins, reported that transglutaminase catalyzes the formation of ε-(γ-glutamyl)lysine crosslinks and the substitution of a variety of primary amines for the γ-carboxyamide groups of protein-bound glutamyl residues. Unlikely, in the present study was observed inconsistency of the gel improvement in chicken and pork sausages. We therefore, recognized in this study that the unique reaction among MHC proteins with MTG could be peculiar reaction indeed. In addition, it is considered as a beneficent substrate in gelation and facilitates the development in texture of chicken and pork sausages as reported in a previous study (Ahhmed et al., 2005). Industrially, the outcomes of this study can contribute to the improvement in the texture of various meats, especially those treated mechanically and to avoid some undesirable meat states (i.e. meat pasting, softening, or stickiness) to meet the demands of consumers. Further studies have to be conducted to specify the exact required amount of MTG to eschew any misuse or misemploy of MTG.

CONLUSION

As far as we know these findings in recognizing the inconsistency in texture improvement of chicken and pork sausages employing MTG as a protein-binding agent are the first of its type. This study provides a discriminative data that contribute to support our hypothesis that MTG inconsistently improves the texture of meats. Furthermore, these findings also show a marked variation in the shear force of chicken and pork samples, which originally might relate to the G-L and collagen content or to the variation in morphogenesis of the muscles in both meat types. We therefore conclude that MTG has a distinctive feature and peculiar reaction occurs with meat proteins and recurs to the type, origin and condition of the animal muscle.

REFERSENCS

Ahhmed, A. M., S. Kawahara, T. Soeda and M. Muguruma. 2005. The improvement of gel strength of sausages prepared from three types of meat with microbial transglutaminase. In proceeding of the 51st International congress of meat science and technology. pp. 54, 7-12 August 2005, Baltimore, Maryland, USA.

Ahhmed, A. M., N. Matsumoto, S. Kawahara, K. Ohta, R. Kuroda, T. Okayama, K. Nakade, M. Numata, T. Nakamura and M. Muguruma. 2006. Effects of various treatments on the texture softening of post-breeding mature cow's meat. In proceeding of the 52nd International congress of meat science and technology. pp. 431-432, 13-18 August 2006, Dublin, Ireland.

Ando, H., M. Adachi, K. Umeda, A. Matsuura, M. Nonaka, K. Uchio, H. Tanaka and M. Motoki. 1989. Purification and characteristics of a novel transglutaminase derived from microorganisms. Agric. Biologic. Chem. 53:2613-2617.

Chanyongvorakul, Y., Y. Matsumura, M. Nonaka, M. Motoki and T. Mori. 1995. Physical properties of soy bean and board bean 11S globulin gels formed by transglutaminase reaction. J. Food Sci. 60:483-488, 493.

Dimitrakopoulou, M. A., J. A. Ambrosiadis, F. K. Zetou and J. G. Bloukas. 2005. Effects of salt and transglutaminase (TG) level and processing conditions on quality characteristics of phosphate-free, cooked, restructured pork shoulder. Meat Sci. 70:743-749.

Erwanto, Y., S. Kawahara, K. Katayama, S. Takenoyama, H. Fujino, K. Yamauchi, T. Morishita, Y. Kai, S. Watanabe and M. Muguruma. 2003. Microbial transglutaminase modifies gel properties of porcine collagen. Asian-Aust. J. Anim. Sci. 15:1204-1209.

Erwanto, Y., S. Kawahara, K. Katayama, M. A. Ahhmed, K. Yamauchi, K. B. Chin and M. Muguruma. 2005. Effect of existence of exogenous protein on physicochemical properties of heat- and transglutaminase-induced bovine collagen-peptide gel. J. Food Sci. 70:E505-E509.

Gornall, A. G., C. J. Baradawill and M. M. David. 1949. Determination of serum protein by means of the biuret reaction. J. Biologic. Chem. 177:751-766.

Jiang, S. T., J. F. Hsieh and G. J. Tsai. 2004. Interactive effects of microbial transglutaminase and recombinant cystatin on the mackerel and hairtail muscle protein. J. Agric. Food Chem. 52:3617-3625.

Katayama, K., K. B. Chin, S. Yoshihara and M. Muguruma. 2006. Microbial transglutaminase improves the properties of meat protein and sausages texture manufactured with low-quality pork loin. Asian-Aust. J. Anim. Sci. 19:102-108.

Kumazawa, Y., K. Seguro, M. Takamura and M. Motoki. 1993. Formation of ε-(γ-glutamyl) lysine cross-link in cured horse mackerel meat induced by drying. J. Food Sci. 58:1062-1064.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227:680-685.

Matheis, G. and J. Whitaker. 1987. A review: Enzymatic cross-linking of proteins applicable to food. J. Food Biochem. 11:309-327.

Muguruma, M., M. A. Ahhmed, Y. Namaba, S. Kawahara, K. Ohta, H. Tanabe, R. Kuroda, K. Nakade, M. Numata and T. Nakamura. 2006. Development of a new soft chicken loaf particularly for elderly persons and infants. In proceeding of the 52nd International congress of meat science and technology. pp. 539-540, 13-18 August 2006, Dublin, Ireland.

Muguruma, M., K. Tsuruoka, H. Fujino, S. Kawahara, K. Yamauchi, S. Matsumura and T. Soeda. 1999. Gel strength enhancement of sausages by treating with microbial transglutaminase. In proceeding of the 45th International congress of meat science and technology. pp. 138-139, 1-6 August 1999, Yokohama, Japan.

Muguruma, M., K. Tsuruoka, K. Katayama, Y. Erwanto, S. Kawahara, K. Yamauchi, S. K. Sathe and T. Soeda. 2003. Soybean and milk protein modified by transglutaminase improves chicken sausage texture even at reduced levels of phosphate. Meat Sci. 63:191-197.

Muguruma, M., T. Nishimura, R. Umetsu, I. Goto and M. Yamauchi. 1987. Humectants improve myosin extractability and water activity of raw, cured intermediate moisture meats. Meat Sci. 20:179-194.

Nonaka, M., H. Tanaka, A. Okiyama, M. Motoki, H. Ando, K. Umeda and A. Matsuura. 1989. Polymerization of several proteins by Ca^{2+}-indepentent transglutaminase derived from microorganisms. Agric. Biologic. Chem. 53:2619-2623.

Nio, N., M. Motoki and K. Takinami. 1986. Gelation mechanism of protein solution by transglutaminase. Agric. Biologic. Chem. 50:851-855.

Pietrasik, Z. 2003. Binding and textural puppetries of beef gels processed with κ-carrageenan, egg albumin and microbial transglutaminase. Meat Sci. 63:317-324.

Poulanne, E. J. and R. N. Terrell. 1983. Effects of salt levels in prerigor blends and cooked sausages on water binding, released fat and pH. J. Food Sci. 48:1022-1024.

Ruiz-Carrascal, J. and J. Regenstein. 2002. Emulsion stability and water uptake ability of chicken breast muscle proteins as affected by microbial transglutaminase. J. Food Sci. 67:734-739.

Sakamoto, H., Y. Kumazawa, K. Seguro, T. Soeda and M. Motoki. 1995. Gel strength enhancement by addition of microbial transglutaminase during onshore surimi manufacture. J. Food Sci. 60:300-304.

Tseng, T. F., D. C. Liu, M. T. Chen. 2000. Evaluation of transglutaminase on the quality of low-salt chicken meat-balls. Meat Sci. 55:427-431.

Tsukamasa, Y., K. Sato, Y. Shimizu, C. Imai, S. Sugiyama, Y. Minegishi and M. Kawabata. 1993. ε-(γ-glutamyl)lysine crosslink formation in sardine myofibril sol during setting at 25°C. J. Food Sci. 58:785-787.

Yokoyama, K., T. Ohtsuka, C. Kuraishi, K. Ono, Y. Kita, T. Arakawa and D. Ejima. 2003. Gelation of food protein induced by recombinant microbial transglutaminase. J. Food Sci. 68:48-51.