

Microbial Transglutaminase Improves the Property of Meat Protein and Sausage Texture Manufactured with Low-quality Pork Loins

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ABSTRACT : Microbial transglutaminase (MTGase) was investigated to determine whether it was an effective binding agent for the processing of low-quality pork loins. MTGase especially promoted the coagulation of myosin heavy chain (MHC). However, the effect of MTGase on MHC from low-quality meat was less than that from the normal meat when the reaction time was not enough. The breaking strength of the heat-induced gel made of myosin B from low-quality meat with MTGase was lower than that of normal meat. Sausage made with low-quality meat with MTGase did not exhibit improved hardness, as compared to that made with normal meat. Results of this study indicated that use of low-quality meat in the manufacture of sausage was feasible to get textural property equal to that of normal meat sausage, when a half or more of the raw material was normal meat and MTGase was used in the sausage. (*Asian-Aust. J. Anim. Sci.* 2006. Vol 19, No. 1 : 102-108)

Key Words : Microbial Transglutaminase, Low-quality Pork Loin, Sausage Texture

INTRODUCTION

Pale, soft, exudative (PSE) pork is one of the low-quality meats and is not adequate as fresh meats or as raw meat materials for the manufacture of processed meat products (Okitani, 1996). This has been already confirmed by numerous groups; for example, a rapid drop of pH may induce rapid sugar-metabolism, or a high body temperature post slaughter and irregularly rapid rigor mortis may result in the production of denatured muscle proteins. These phenomena are found in stress-sensitive livestock, especially pork, as a result of porcine stress syndrome and malignant hyperthermia syndrome caused by irregular muscle-metabolism brought on by some kinds of anesthetics, or unsuitable control of pre- and post slaughter conditions (Penny, 1967; Yasui et al., 1973). Thus, proteins extracted from PSE pork are severely denatured at an early stage of postmortem. In addition, physicochemical properties of PSE pork are inferior to normal meat in terms of color, rheological property, and chemical components. Although Japan recently imports a large amount of pork from Denmark for meat products, a part of frozen-imported pork loins show low quality like PSE. But, no proper processing technique for the use of these meats can be satisfied with consumers for their demands.

Incorporation of covalent bonds into the spaces between protein molecules or within the molecules is considered to

be effective in improving the texture of meat products. The use of transglutaminase (TGase) is one method for the improvement of textural characteristics (Kuraishi et al., 1997; Motoki and Seguro, 1998; Erwanto et al., 2002; Chin and Chung, 2003; Muguruma et al., 2003). TGase promotes protein coagulation by catalyzing the cross-links between glutamine and lysine residues. These cross-links improve the viscoelasticity of protein gel and form a gel without heating. TGase is also highly correlated with the coagulation of blood in our body, which depends on calcium ions, and microbial TGase (MTGase, EC 2.3.2.13) made by microorganisms is calcium independent and excellent in the functional property for utilization (Ando et al., 1989). Consequently, MTGase has been widely used to improve the textural quality of several foods, such as kamaboko, ham, sausage, tofu, and noodles (Motoki and Seguro, 1998). Although previous studies have been performed for various native proteins as substances to react with MTGase, no information whether MTGase can work with low-quality meat has been observed.

Thus, the objectives of this study were to investigate whether MTGase could be used as an effective binding agent for low-quality pork, to evaluate the reactivity of MTGase to myosin B (actomyosin) or water-soluble protein from this meat, and to determine the efficacy of MTGase on the textural properties of low-fat sausages manufactured with this meat.

MATERIALS AND METHODS

Preparation of myosin B, water-soluble protein and myosin from porcine meat

Normal and low-quality pork loins (*Longissimus dorsi*)

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Table 1. Physicochemical properties of normal and low-quality meats

	Normal meat	Low-quality meat
Pork color standard value	5	1
pH	5.97±0.08	5.42±0.10
L*	48.1±1.9	59.8±1.6
Moisture content (%)	73.4±0.3	72.4±0.2
Protein content (%)	22.9±0.3	24.1±0.7

Means±SEM.

Surface L* was measured 7 times for each meat, and the other parameters except PCS value were measured 3 times for each meat.

imported from Denmark were used for the experiment. After about 6 months of storage at -20°C, pork loins were thawed at 4°C and classified into normal and low-quality meats based on their surface color and pH. The pH of each meat was measured by a pH meter (F-14; Horiba Ltd., Kyoto, Japan). Moisture and protein contents of raw meats were measured by dry-oven and Kjeldahl methods, respectively (Yasui and Aoyagi, 2000). To classify the meats, we measured the whiteness (L values) using a Hunter Lab scan spectrophotometer (CM1000, Minolta Co., Ltd., Osaka, Japan). The pork color standard (PCS) value was defined using the Japanese pork color standard (Nakai et al., 1975; Macdougall, 1994). Data for raw meats are shown in Table 1. After the removal of fats and connective tissues, pork loins were minced with a 5-mm plate, vacuum-packaged, stored at -20°C for a week, and thawed at 4°C before experiments. This second storage at -20°C was performed supposing the worst conditions in meat processing.

Myosin B was extracted by the method of Szent-Györgyi (1951). The minced meats were added to 3 volumes of Weber-Edsall solution (0.6 M KCl, 0.04 M NaHCO₃, 0.01 M Na₂CO₃, 1 mM NaN₃) and stood at 4°C for 40 h with occasional and mild stirrings. Next, we added 2 volumes of 0.6 M KCl, stirred the mixture well, and centrifuged it at 30,000 g for 1 h. The supernatants were collected and diluted with 14 volumes of cold water, stirred well, and centrifuged at 12,000 g for 30 min. After the last centrifugation, we collected the precipitants as Myosin B, added NaCl to 0.5 M and NaN₃ to 2 mM into them, and stored them at 0°C for a few days until analyzed. Water-soluble protein (WSP) was extracted with physiological saline (10 mM Na-Phosphate, 0.8% NaCl, pH 7.5). The minced meats were homogenized with 2 volumes of physiological saline by a Polytron mixer (Kinematica Co. Ltd., Switzerland), and the supernatants as WSP were collected after centrifugation at 12,000 g for 15 min. Crude myosin was prepared using the method of Margossian and Lowey (1982). The minced meats were added to 3 volumes of Guba-Straub solution (0.3 M KCl, 0.1 M KH₂PO₄, 0.05 M K₂HPO₄, 1 mM NaN₃), and stood for 30 min after 5 min of stirring. Then, they were centrifuged at 30,000 g for 15

min and the supernatants were collected as crude myosin.

Protein concentration of extracted protein solution was determined using biuret method (Gornal et al., 1949) using bovine serum albumin as a standard. These proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 15% acrylamide gel or a gradient (5-15% acrylamide) slab gel for separating proteins (Laemmli, 1970). Protein bands were stained with Coomassie Brilliant Blue R-250 (CBB).

Reaction of myosin B, WSP, or myosin with MTGase

MTGase was obtained as a purified enzyme from Ajinomono Co. Ltd. (Tokyo, Japan) and was dissolved in 10 mM NaCl. For SDS-PAGE analysis, myosin B (2 mg/ml), including 0.6 M NaCl and 50 mM imidazole-HCl (pH 6.0), was incubated with or without 0.01 mg/ml of MTGase. The procedure for crude myosin was the same as that for myosin B. However, physiological saline was used for WSP, instead of 0.6 M NaCl. The ratio of enzyme to substrate was 1/200 (w/w). After incubation at 37°C for 2 h (0, 3, and 20 min for crude myosin), the reaction mixtures were mixed with an equal volume of SDS-PAGE sample buffer and heated at 95°C for 5 min. A gradient gel (5-15% acrylamide) was used for separating proteins (Laemmli, 1970). Protein bands were stained with CBB. For breaking strength analysis, myosin B (15 mg/ml in 0.5 M NaCl) was mixed with or without MTGase (0.075 mg/ml). The ratio of enzyme to substrate was 1/200 (w/w). These mixtures were pipetted into a 96-well flat-bottomed microplate, 350 µl in each well, with care taken to avoid entrapment of air bubbles (Sakamoto et al., 1994). After incubation at 37°C for 1 h, this plate was heated at 75°C for 30 min to inactivate the enzyme and cooled at 0°C for 18 h until analyzed. A creep meter (RE2-33005S, Yamaden Co. Ltd., Tokyo, Japan) and a sample-height counter (HC2-33005S, Yamaden) were used for breaking strength analysis. A globular plunger with 5-mm diameter was used, and the sample was compressed with a table speed of 1 mm/sec.

Model sausage test

Model sausages were prepared with 1.7% NaCl and 0.3% sodium tripolyphosphate (STPP) and with or without 0.001% of MTGase. Each value was for the final weight of the sausage batter, which was adjusted with cold water to 140% of the weight of the original meat. Activa TG-S (Ajinomono), which included 1% of MTGase and 10% of STPP, was used as a source of MTGase. Sausage batter was prepared as finely ground paste by 2 min mixing with a Speed-cutter (MK-K45; National Co. Ltd., Osaka, Japan). Treatments tested were normal meat (N), low-quality meat (L), low-quality meat: normal meat by 1:1 (L+N), and low-quality meat+6.7% of myosin B paste extracted from

Table 2. Composition of sausage made of normal or low-quality meat

Components	Amount (g)			
	N	L	L+N	L+NB
Normal meat	100.0	-	50.0	-
Low-quality meat	-	100.0	50.0	93.3
N-Myosin B	-	-	-	35.0
NaCl	2.4	2.4	2.4	2.4
Sodium triphosphate	0.4	0.4	0.4	0.4
H ₂ O	37.2	37.2	37.2	8.9
Total	140.0	140.0	140.0	140.0

N-Myosin B: myosin B paste with 4.4% protein extracted from normal meat.

N, L, L+N, and L+NB: unheated sausage made of normal meat, low-quality meat, low-quality meat+50% of normal meat, and low-quality meat + 6.7% of myosin B extracted from normal meat, respectively.

All sausages were prepared with (+) or without (-) MTGase.

normal meat (L+NB) (Table 2). The protein concentration of this replaced myosin B was 4.4% and, consequently, the replaced protein was 1.54% of meat. Each type of sausage batter was stuffed into a polyvinyl-chloride casing (25 mm diameter). Sausages were held at 37°C for 2 h for MTGase reaction, heated at 75°C for 40 min in a water bath, and cooled in ice water for 30 min. To evaluate cooking loss, sausages and casings were weighed. Then, the sausages were wrapped with polyvinyl chloride film and held at 4°C for 17 h.

A portion of each sausage batter was held at 37°C for 2 h for MTGase reaction and then stored at 0°C to evaluate the effect of MTGase at low temperature. After 2 days, protein was extracted from these unheated sausages. The sausages were homogenized with 9 volumes of solution (0.5 M NaCl, 6 M Urea) by a Polytron mixer, and the supernatants were collected after centrifugation at 30,000 g for 15 min. Protein concentration was determined with biuret method (Gornall et al., 1949) using bovine serum albumin as a standard. These proteins were analyzed by SDS-PAGE using gradient (5-15% acrylamide) slab gel for separating proteins (Laemmli, 1970), and protein bands were stained with CBB.

The same apparatuses described above were used for measuring sausage texture (Bourne, 1978). For texture profile analysis, sausages were prepared to adequate size (bottom: 10×5 mm, height: 10 mm) using a thin knife, and held at 25°C for 15 min before measurement. A columnar plunger with a 20 mm diameter was used, and compressed samples with a plunger speed of 1 mm/sec. It compressed the sample twice to 80% of its original height.

Statistical analysis

Data were analyzed by analysis of variance using the SPSS program for Windows. When significant differences were observed ($p < 0.05$) among treatments, Tukey's HSD (honestly significant difference) test was performed.

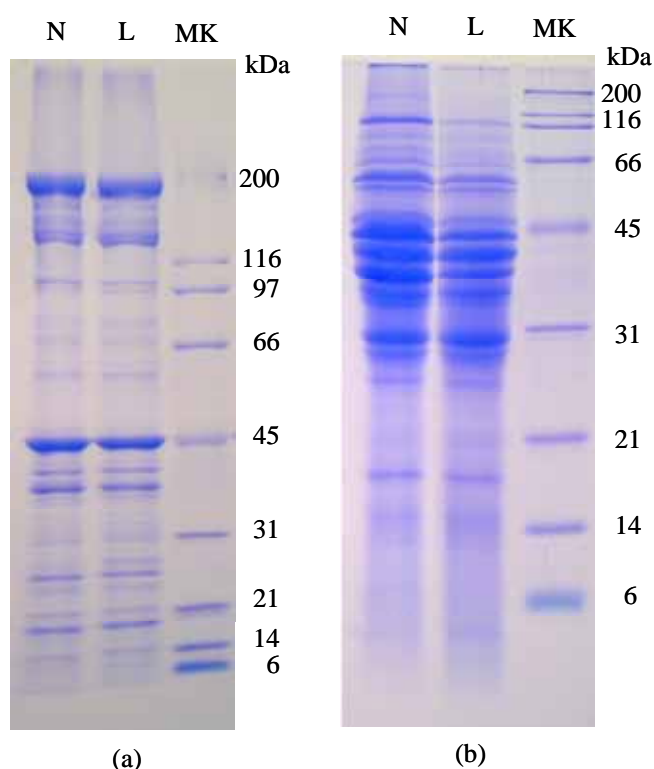


Figure 1. SDS-PAGE of extracted proteins from normal or low-quality meats. N: normal meat, L: low-quality meat, MK: molecular weight marker. Applied protein is 25 µg to each lane. (a) Myosin B. A gradient (5-15% acrylamide) gel was used for separation. (b) Water-soluble protein. A homogeneous (15% acrylamide) gel was used for separation.

RESULTS AND DISCUSSION

Extraction yield and protein profiles of myosin B and WSP

The extraction yield for each protein varied depending on the quality of proteins. The yields of myosin B extracted from normal and low-quality meats were 13.3% and 7.3%, respectively. This showed the extraction of myosin B from low-quality meat was more difficult than that from the normal counterpart. On the other hand, the yields of WSP from normal and low-quality meats were 2.8% and 3.5%, respectively. We thought this result was partially due to the relatively high water-holding capacity of normal meat. That is to say, free water including WSP of normal meat was less than that of low-quality one. Although the pH value of porcine muscle right after slaughter is ordinarily near 7.2, it falls to 5.5 at rigor mortis when lactic acid accumulates by anaerobic glycolysis (Okitani, 1996), and meat proteins are easily denatured by the low pH. Consequently, differences of extraction yield for meat proteins between normal and low-quality meats might be caused by the different degrees of protein denaturation.

SDS-PAGE profiles of myosin B and WSP are shown in Figure 1. In the protein profiles of myosin B, there are no

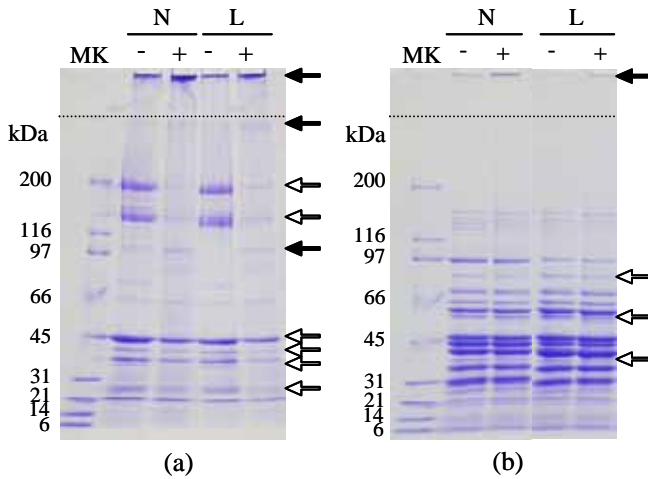


Figure 2. SDS-PAGE of WSP or myosin B incubated with (+) or without (-) MTGase. N: normal meat, L: low-quality meat, MK: molecular weight marker. Applied protein is 25 μ g to each lane. Closed and open arrows show the increased and decreased bands by MTGase treatment, respectively. Dotted lines show the borders between the stacking (upper) and separating (lower) gels. (a) Myosin B. (b) Water-soluble protein.

major but some minor differences between two meats (Figure 1a). This result may support the previous report that the component of myosin B from normal meat was different from that of PSE counterpart (Park et al., 1975). In WSP, protein bands containing the MWs of about 100 and 60 kDa in low-quality meat did not show clearly, as compared to the normal counterpart in SDS-PAGE (Figure 1b). This difference might be partially due to more drip loss of WSP from low-quality meat, which had a lower water-holding capacity than normal one during freezing and thawing.

Reaction of extracted protein and MTGase

SDS-PAGE analysis was performed to determine if the reaction of MTGase to myosin B or WSP was different from normal and low-quality meats (Figure 2). In the case of myosin B, no differences were observed between the two meats. Myosin heavy chain (MHC, MW 200 kDa) almost disappeared after MTGase treatment in myosin B extracted from both normal and low-quality meats (Figure 2a). The protein band, which had very high MW and could not enter into the stacking gel, tended to increase after MTGase treatment for 2 h, probably because the proteins coagulated each other by MTGase and became a biopolymer with ultra-high molecule weight. The amount of actin (42 kDa) was found to decrease after MTGase treatment in our study, even though MTGase rarely reacted to actin (Sakamoto et al., 1994). As previously described (Tseng et al., 2002), actin also might be incorporated into a biopolymer mainly made of myosin, which had high reactivity to MTGase. Although WSP rarely reacted with MTGase, some protein bands were decreased and a biopolymer was formed by

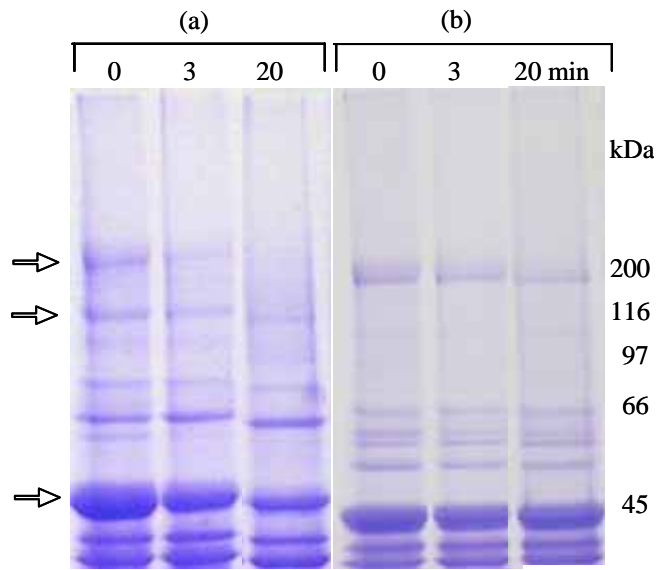


Figure 3. SDS-PAGE of crude myosin incubated with MTGase for 0, 3 or 20 min. Arrows show the decreased bands by MTGase treatment. (a) Myosin from normal meat. (b) Myosin from low-quality meat.

MTGase treatment as a result (Figure 2b). However, no major differences in WSP were observed in SDS-PAGE profiles between normal and low-quality meats.

Crude myosin extracted with Guba-Straub solution was reacted with MTGase to determine whether MHC was reacted with MTGase relatively for a short time. The SDS-PAGE pattern showed MHC from normal meat started to decrease after 3 min treatment and the MHC band was almost disappeared after 20 min (Figure 3a). On the other hand, MHC from low-quality meat did not decrease after 3 min of MTG treatment, but decreased almost after 20 min (Figure 3b). Some of the other proteins decreased more easily by MTGase treatment of crude myosin from normal meat, as compared to low-quality one. Consequently, the reactivity of crude myosin from low-quality meat to MTGase was observed to be inferior to that from normal counterpart.

Since a rapid decrease of MHC was found in both normal and low-quality meats after MTGase treatment for 20 min, MTGase was expected to give a good binding effect and heat-induced gel forming capacity, which is important in the meat processing (Ishioroshi, 1996). However, since the reactivity of MTGase to low-quality meat seems to be inferior to that of normal counterpart, the binding capacity of low-quality meat, even if processed with MTGase, might be lower than that of normal counterpart.

Effect of MTGase on heat-induced gel formation of myosin B

Because MTGase mainly reacted to MHC and therefore

Table 3. Gel strength of heat-induced gels of myosin B from normal and low-quality meats

	Gel strength (N)	
	-MTGase	+MTGase
Normal	0.0045 ^a	0.1381 ^b
L	0.0046 ^a	0.1125 ^c

Means (n = 16).

Means with different letters are statistically different (p<0.05).

Table 4. Hardness values of sausages as affected by addition of MTGase and quality of meats

	Hardness (N)	
	-MTGase	+MTGase
Normal	3.99 ^a	7.10 ^b
L	1.95 ^c	2.63 ^d
L+N	2.96 ^{de}	3.97 ^a
L+NB	2.47 ^{cd}	3.27 ^e

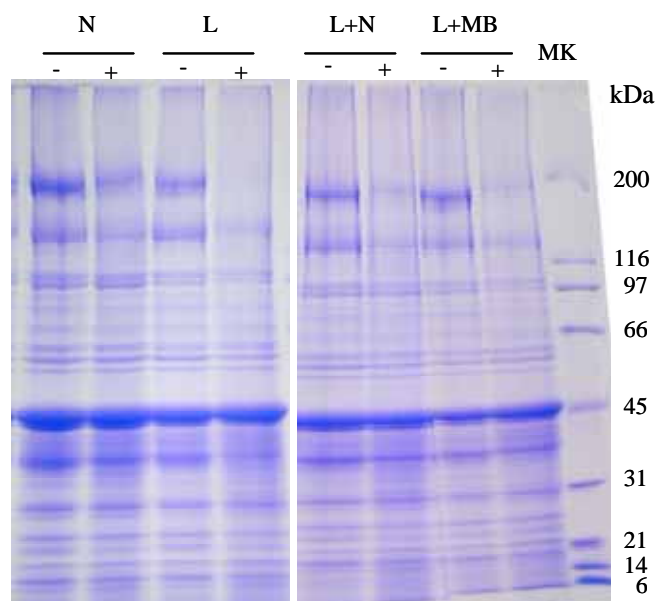
Means (n = 8).

Means with different letters are statistically different (p<0.05).

was considered to positively effect on the heat-induced gel formation of protein in meat, its effect on myosin B extracted from normal and low-quality meats was respectively investigated. The gel strengths of heat-induced gel of myosin B are shown in Table 3. MTGase in this study reacted to myosin B and significantly (p<0.05) improved its heat-induced gel strength. This result supports the previous report by Numata et al. (1989), who reported that the gel strength of myosin B from normal meats remarkably increased with MTGase treatment. However, the gel strength of the gel made of myosin B from low-quality meat with MTGase was significantly (p<0.05) lower than that of normal one. This result indicated that myosin B from low-quality meat had somewhat less reactivity to MTGase than that from normal one as shown in Figure 3. Thus, the utilization of myosin B from low-quality meat was suggested to be inferior to that from normal one.

Effect of MTGase on SDS-PAGE profile and rheological property of sausage

SDS-PAGE profiles of proteins extracted from unheated sausages (meat batter) showed that several bands decreased by treatment with MTGase (Figure 4). The protein bands with MWs of 200 (MHC) and approximately 120 kDa were decreased markedly after treatment with MTGase. These proteins appeared to have coagulated into a biopolymer in response to MTGase, as described previously. The decrease of proteins by MTGase was shown in all treatments including the test for replacing low-quality meat to normal one or myosin B solution. Therefore, MTGase was considered to react with low-quality meat as well as normal meat in unheated sausages. Since MTGase remarkably reacted to MHC, as described in the case of myosin B, MTGase treatment was expected to improve the rheological property of the sausage by improvement of its heat-induced

**Figure 4.** SDS-PAGE of proteins extracted from unheated sausages prepared with (+) or without (-) MTGase. N, L, L+N, and L+MB: unheated sausage made of normal meat, low-quality meat, low-quality meat+50% of normal meat, and low-quality meat+6.7% of myosin B extracted from normal meat, respectively. MK: molecular weight marker.

gel forming property.

We measured the rheological property of sausages made with low-quality and normal meats to determine whether the property was affected by the reaction with MTGase. The hardness of the sausage made of normal meat improved (p<0.05) by MTGase treatment, and those of other tests also improved (Table 4). The hardness of the sausage made of normal meat with MTGase was the best, and the other sausage types could not obtain a similar degree of hardness. These results suggested that MTGase could react to normal meat or the combination of normal and low-quality meats, but something that obstructed the heat-induced gel formation resulted in less hardness of low-quality meat sausage compared to normal meat, even when low-quality meat sausages were made with MTGase. The low-quality meat sausage made with a half of normal meat and MTGase showed its hardness was as same as that of normal one without MTGase, and showed the possibility of the usage of low-quality meat equal to normal one. The low-quality meat sausage made with myosin B from normal meat showed less improvement of hardness than the other tests. In this case, because the added myosin B was a solution with high salt concentration, MTGase was considered to preferentially react to the myosin B and consequently not to react to low-quality meat well. In addition, because the structural proteins, such as collagen, were decreased by reduction of meat with the addition of myosin B to low-quality meat, the hardness of sausage was not improved as much as that of

Table 5. Cooking loss of sausages as affected by addition of MTGase and quality of meats

	Cooking loss (%)	
	-MTGase	+MTGase
Normal	2.80 ^a	4.51 ^a
L	11.95 ^b	12.66 ^b
L+N	7.05 ^c	8.95 ^c
L+NB	7.20 ^c	9.14 ^c

Means (n = 3).

Means with different letters are statistically different (p<0.05).

normal counterpart. In any event, hardness values were consistent with the evaluation by the organoleptic assessment, and the discussion using them was considered to be meaningful. These results indicated that MTGase could react with normal and low-quality meats.

The amount of cooking loss was the least (p<0.05) in the sausage made of normal meat and the most (p<0.05) in that made of low-quality meat (Table 5). The cooking loss of sausages with low-quality meat was decreased by the combined use of normal meat or myosin B, and it appeared that the amount of cooking loss was affected by the quality of meat protein. The cooking loss tended to increase by MTGase treatment in all cases, possibly because the space to hold water in protein network decreased as a result of the increased cross-linking by MTGase.

Low-quality meat showed less property than normal one, such as the extraction yield of myosin B, the components of extracted protein, and the reactivity of myosin B with MTGase. Thus, the sausage made of the low-quality meat showed less hardness value and more cooking loss than that of normal one. But, MTGase treatment improved these properties of low-quality meat except for cooking loss, which was improved by combined use with normal meat or myosin B. The low-quality meat sausage made with a half of normal meat and MTGase showed its hardness was as same as that of normal one without MTGase, and showed the possibility of the usage of low-quality meat equal to normal one.

REFERENCES

- Ando, H., M. Adachi, K. Umeda, A. Matsuura, M. Nonaka, R. Uchio, H. Tanaka and M. Motoki. 1989. Purification and characteristics of a novel transglutaminase derived from microorganisms. *Agric. Biol. Chem.* 53:2613-2617.
- Bourne, M. C. 1978. Texture profile analysis. *Food Technol.* July. 62-66.
- Chin, K. B. and B. K. Chung. 2003. Utilization of transglutaminase for the development of low-fat, low-salt sausages and restructured meat products manufactured with pork ham and loins. *Asian-Aust. J. Anim. Sci.* 16:261-265.
- Erwanto, Y., M. Muguruma, S. Kawahara, T. Tsutsumi, K. Katayama, K. Yamauchi, T. Morishita, Y. Kai and S. Watanabe. 2002. Effect of heating on polymerization of pig skin collagen using microbial transglutaminase. *Asian-Aust. J. Anim. Sci.* 15:1204-1209.
- Gornall, A. G., C. J. Bardawill and M. M. David. 1949. Determination of serum protein by means of the biuret reaction. *J. Biol. Chem.* 177:751-766.
- Ishioroshi, M. 1996. *Shokuniku Seibun no Kako Tokusei.* (Ed. A. Okitani). *Niku no Kagaku*, pp. 131-137. Tokyo, Japan: Asakura shoten (in Japanese).
- Kuraishi, C., J. Sakamoto, K. Yamazaki, Y. Susa, C. Kuhara and T. Soeda. 1997. Production of restructured meat using microbial transglutaminase without salt or cooking. *J. Food Sci.* 62:488-490, 515.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227:680-685.
- Macdougall, D. B. 1994. Color of Meat. In: (Ed. A. M. Pearson, and T. R. Dutson), *Quality attributes and their measurement in meat, poultry and fish products.* Adv. Meat Res., 9. Blackie Academic & Professional an imprint of Chapman & Hall Glasgow, UK.
- Margossian, S. S. and S. Lowey. 1982. Preparation of myosin and its subfragments from rabbit skeletal muscle. (Ed. D. W. Frederiksen and L. W. Cunningham). *Methods Enzymol.*, Vol. 85, pp. 55-59. New York: Academic Press.
- Motoki, M. and K. Seguro. 1998. Transglutaminase and its use in food processing. *Trends in Food Science and Technology*, 9:204-210.
- Muguruma, M., K. Tsuruoka, K. Katayama, Y. Erwanto, S. Kawahara, K. Yamauchi, S. K. Sathe and T. Soeda. 2003. Soybean and milk proteins modified by transglutaminase improves chicken sausage texture even at reduced level of phosphate. *Meat Sci.* 63:191-197.
- Nakai, H., F. Saito, T. Ikeda, S. Ando and A. Komatsu. 1975. Standard models of pork colour. In: *Bulletin of the National Institute of Animal Industry*, 29:69-74.
- Numata, M., H. Yamada, T. Nakamura and M. Muguruma. 1989. The effect of transglutaminase on heat-induced gelation and water holding capacity of myosin B. *Nippon Shokuhin Kogyo Gakkaishi*, 36:832-838 (in Japanese).
- Okitani, A. 1996. *Shokuniku no Oishisa to Jukusei.* (Ed. A. Okitani). *Niku no Kagaku*, pp. 85. Tokyo, Japan: Asakura shoten (in Japanese).
- Park, K. H., M. Muguruma, T. Fukazawa and T. Ito. 1975. Relationship between superprecipitating activity and constituents of myosin B prepared from normal and PSE porcine muscle. *Agric. Biol. Chem.* 39:1363-1370.
- Penny, R. W. 1967. The effect of post-mortem conditions on the extractability and adenosine triphosphatase activity of myofibrillar proteins of rabbit muscle. *J. Food Technol.* 2:325-338.
- Sakamoto, H., Y. Kumazawa and M. Motoki. 1994. Strength of protein gels prepared with microbial transglutaminase as related to reaction conditions. *J. Food Sci.* 59:866-871.
- Szent-Györgyi, A. 1951. *Chemistry of muscle contraction*, 2nd ED, pp. 151-152. New York: Academic Press.
- Tseng, T. F., M. T. Chen and D. C. Liu. 2002. Purification of transglutaminase and its effects on myosin heavy chain and actin of spent hens. *Meat Sci.* 60:267-270.

- Yasui, A. and Y. Aoyagi. 2000. Measurement of Food Components. (Ed. T. Sugahara and A. Maekawa). Handbook of Food Analysis, pp. 15-32. Tokyo, Japan: Kenpakusha (in Japanese).
- Yasui, T., T. Gotoh and J. Morita. 1973. Influence of pH and temperature on properties of myosin A in glycerol-treated fiber bundles. *J. Agric. Food Chem.* 21:241-246.