Transglutaminase polymerizes meat proteins at -35°C and may have industrial applications as a biological protective film

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Abstract— Over the last three decades, microbial transglutaminase (MTG) has been used as an additive to aggregates proteins and produce low-salt meat products. The addition of salt or MTG to meat products has favourable effects on the textural properties. However, many people, especially the hypertensive and elderly, avoid products with high salt, motivating the search for alternatives. The functional properties of MTG particularly its ability to interlock proteins through glutamine and lysine amino bonds, suggests it could be a pivotal element in improving mechanically processed meat products. The optimum temperatures for creating polymers is from 30-55°C. This study provides evidence that MTG works at low and even freezing temperatures. This work aimed to determine if MTG was active at -35°C. Surprisingly, MTG polymerized beef proteins at -35°C during 35 days of storage, and had the potential to decrease hardness, reduce weight loss, and protect against colour deterioration. SDS-PAGE showed that the proteins were denatured when treated with MTG. The strong activity of MTG suggests this enzyme could be an important functional tool, and could benefit the food industry as a preservative material, a biological protective film, or even have medical applications in the near future.

Keywords: Transglutaminase, biological protective film, meat quality, frozen meat.

I. INTRODUCTION

Transglutaminase is a food additive that has been used in the food industry to promote protein aggregation to improve textural properties. MTG helps accelerate protein aggregation at warm temperatures (30-55°C), improving the texture and appearance of meat products. Whether MTG works at other temperatures is unknown, but this study provides evidence that MTG works at freezing temperatures. Freezing is a widely accepted preservation method that is used to store meat for long periods of time (Pietrasik, Janz, 2009). Frozen meat products are rated for appearance, colour, texture, and flavour. Meats should be properly and carefully wrapped in moisture- or vapour-proof materials, ensuring that no chilled air enters, and moisture is not allowed to evaporate. Freezer burn occurs when meats that are being stored at freezing temperatures lose moisture, resulting in greyish-black spots or patches on the surface of meat blocks. These splotches are caused by the evaporation of water molecules as ice, from the surface of the product, or by moisture loss. The goal of this study was to determine if MTG, which is used in the currents study as a coating and preservative for fresh beef steaks and has a variety of functional properties, works at low temperatures. The objective was to determine if MTG is capable of protecting meat frozen at -35°C for 5 weeks, and to find out if MTG catalyzes proteins at freezing temperatures, which could be used in industrial situations. Biological protection films (bio-pro-films), meaning any wrapping material produced by biotechnology using natural materials with no plastic additives or harmful inorganic elements, protects against deterioration or damage to food quality during freezer storage.

II. MATERIALS AND METHODS

The cut of meat used in this study was the *biceps femoris* muscle from Japanese Black Cattle, purchased from a local butchery in Miyazaki, Japan. The meat was vacuum-packaged and stored for 4 days in a chilled refrigerator at 4°C. Meat portions were cut into steaks of approximately 1.2 × 8 × 12 cm, and the visible fatty and connective tissues removed before protein extraction. Polyethylene sheets were used, and other biological agents such as actomyosin extract, MTG, and MTG-actomyosin at 10% and or 30% (w/w), were applied to the samples. All treated samples were placed in a freezer and stored at -35°C for 35 days.

The textural properties, weight loss, molecular weights of extracted proteins, MHC band intensity and colour stability of beef steaks were measured to determine the impact on meat quality of adding stabilizing biological agents (MTG and actomyosin).

III. RESULTS AND DISCUSSION

III.I. *Breaking strength*

Figure 1 shows that MTG reduced the hardness of frozen samples as used in the form of functional coat when incorporated with actomyosin proteins (last two lanes). MTG catalyzed actomyosin proteins that reaction made a bio-pro-film cover around the steak, so samples maintained moisture content, which might have reduced the breaking strength value. However, fresh samples showed a lower value because the marbling in the subjected samples was quite

high and because they were fresh at measurements time. The samples with no wrap had significantly lower values than fresh samples, and significantly lower values than samples wrapped in polyethylene. In samples treated with MTG, the results revealed that myofibrillar proteins reacted with MTG, even at -35°C. However, myofibrillar proteins from the inner samples (2-mm deep) were unchanged, indicating that MTG reacted only with the surface, especially with added actomyosin. Consequently, we suggest that adding MTG to beef steaks stored at -35°C is of great importance, since MTG with added proteins formed a bio-pro-film that protected meat from deterioration.

III.II. *Weight loss*

To test the effect on weight of adding MTG or MTG-actomyosin to samples intended for storage at freezing temperature as bio-pro-film, MTG solution was added to beef steaks at 10% (v/w), and also MTG-actomyosin at 10 and 30%. Sample weight was measured on day 0 and day 35 after freezing, and the differences in weight were calculated. Samples with no wrap lost 5% of their total weight after 35 days of storage (Fig. 2). Sample weight decreased by 4% of total weight for samples wrapped with polyethylene sheets, and 1.8% for samples mixed with actomyosin. WL of samples mixed with MTG was 2.7%, and varied from 1-1.2% in samples treated with a mixture of MTG at 10% or actomyosin at 10 and 30%. These results support the hypothesis that MTG surrounded the meat steaks with a coat that prevented the increase in weight loss. In comparison, WL in samples treated with MTG-actomyosin, was significantly lower than in other samples, especially the non-wrapped samples.

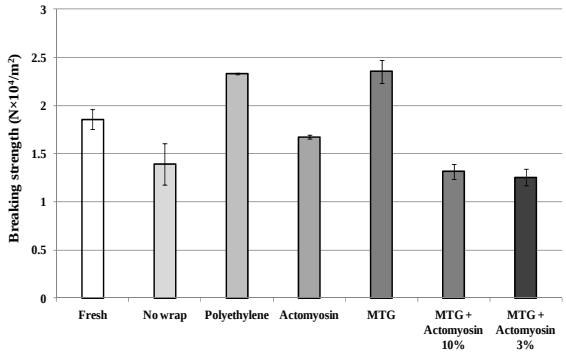

Fig. 1. Breaking strength values of beef steaks treated with different wrapping materials and frozen at -35ºC for 35 days.

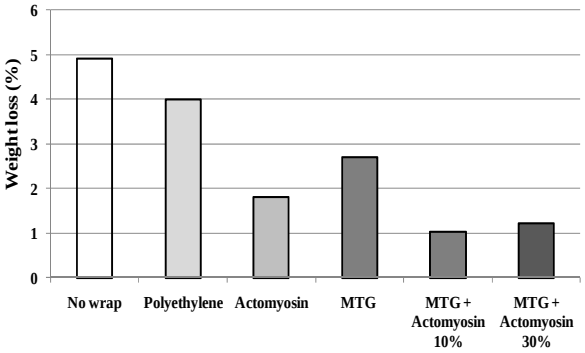

Fig. 2. Weight loss in % of beef steaks treated with different wrapping materials and frozen at -35ºC for 35 days.

III.III. *Protein concentration*

Figure 3 shows protein concentration measurements from samples dissolved in GS-ATP solution from the surface of meat. The values of the fresh samples were significantly higher than for the MTG-treated samples. The impact of 10% MTG on the myofibrils of treated samples was a significant reduction in protein concentration (p <0.01). The concentration of protein in frozen beef decreased with added MTG, and the values were reduced significantly compared to the values for fresh samples. All measurements for other treatments were lower than for MTG-treated samples, implying that the major reaction of MTG was against the MHC proteins, since MTG targets these proteins. Therefore, the reduction in proteins extracted in GS-ATP after addition of MTG solution to beef samples was caused by protein-protein cross-links between MHC proteins. This revealed that MTG catalyses proteins even at freezing temperatures, which could have functional uses in the future.

The protein concentration of inner samples from treated portions stored at -35°C for 35 were also measured (data shown in the poster). These samples sourced from inner part (approximately 2-mm from the surface) and show variable values that are not significantly different from each other. The treatment that gave values similar to the fresh samples was 10% MTG-actomyosin. However, the values for other samples were insignificantly lower than fresh portions, with the exception of samples treated with MTG solution. Freezing and frozen storage of meat can affect the structural and chemical properties of muscle foods, including changes in muscle fibres, and lipid and protein fractions (Pietrasik, Janz, 2009). This supports our hypothesis that MTG with actomyosin may form a bio-pro-film that protects inner proteins, which were found to be very similar to fresh samples. However, our hypothesis states that MTG acted on MHC even at low temperatures, and this is supported by the protein concentration values of surface samples and SDS-PAGE.

III.IV. *MHC changes by SDS-PAGE*

Proteins extracted in GS-ATP from the surface of samples were used for electrophoresis (Fig. 4). The results showed changes in MHC bands that allowed us to observe the protein changes induced by adding MTG to beef steaks. The main reaction was MTG reacting with MHC in myofibrillar proteins (200 kDa), as shown by significant (p < 0.01) reduction in the concentrations of beef proteins. The intensity of MHC bands was clearly reduced with different MTG treatments (Ahhmed, Nasu & Muguruma, 2009). This provided evidence that MTG reacts with MHC proteins in frozen samples. Differences in MHC band intensity with treatment type, and with MTG addition, were presumed to be caused by MTG activity. The reduction in bands size, seen in the SDS-PAGE pattern of samples treated with MTG, were small, indicating that proteins reacted with MTG under freezing conditions (Fig. 3 & table 1). Thus, this indicates that MTG catalyses reactions against meat proteins in both warm environments, and also at low storage temperatures. Band sizes

and other parameters were evaluated with an intensity meter.

III.V. *Evaluation of MHC band intensity*

Table 1 shows values in mm^2/band for MHC band intensity from SDS-PAGE of the surface of treated samples. Band intensity was reduced drastically as MTG solution was added. In comparison to non-wrapped samples, the band intensity in samples treated with MTG solution was reduced by 89%, and with a 74% reduction in samples treated with 10% MTG-actomyosin, and 79% in samples treated with 30% MTG-actomyosin. Of the MTG-treated samples, 10% MTG-actomyosin gave the lowest reduction.

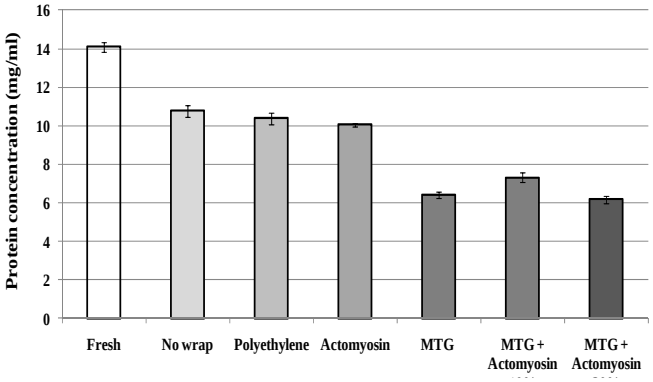

Fig. 3. Concentration in mg/ml of protein in beef steaks treated with different wrapping materials and frozen at -35ºC for 35 days. The proteins were extracted surface of the steaks.

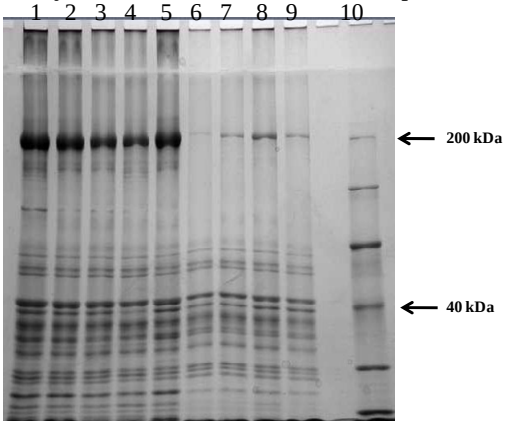

Fig. 4. SDS-PAGE of proteins extracted from beef steaks treated with different wrapping materials and frozen at -35ºC for 35 days

Table 2 shows values for band area from the inner parts of treated samples (SDS-PAGE data not shown). All the treated samples, including non-wrapped samples, showed insignificant differences in their MHC bands, with the exception of the 30% MTG-actomyosin-treated samples. Values for MTG-treated samples with no actomyosin were reduced by 17.5% compared to non-wrapped samples, and 37% for 30% MTG-actomyosin-treated samples. As described above, 10% MTG-actomyosin addition showed the best values, and differed from non-wrapped samples by insignificant percentages. The values of average intensity of the MHC bands from treated samples, showing that non-wrapped, polyethylene, and actomyosin-treated sample band sizes remained unchanged, even though samples treated with 10% MTG solution were almost un-detectable. Samples treated with 10% MTG-actomyosin, or 30% MTG-actomyosin, were reduced drastically compared to the non-wrapped samples, and differed insignificantly from each other. Relative front and the relative quantity of all samples varied, however the variation was based on MTG addition. The relative front (R_F) is the distance of a band from the top of its lane, divided by the total length of the lane (Ahhmed, Kuroda, Kawahara, Ohta, Koji, Toki, & Muguruma, 2009). R_F values for the MHC bands in the samples treated with MTG were higher than for control samples. The increased R_F values indicated that the molecular weights of the MHC bands were reduced in all treated samples, except for samples treated with 10% MTG solution, in which bands were not detected, and could not be evaluated. However, the extractability of MHC was hindered by MTG treatment, implying that the proteins were aggregated and had formed a protein-protein network (PPN).

Table 1. Changes in the size of MHC bands from the surface of beef steaks.

Parameter Size mm^2/band	Sample type					
	No wrap	Polyethylene	Actomyosin	MTG	MTG-actomyosin 10%	MTG-actomyosin 30%
Means	32.40	29.66	31.16	3.59	8.35	6.66
SEM	0.67	2.38	2.33	0.17	0.59	0.68

Table 2. Changes in the size of MHC bands from the inner part of beef steaks.

Parameter Size mm^2/band	Sample type					
	No wrap	Polyethylene	Actomyosin	MTG	MTG-actomyosin 10%	MTG-actomyosin 30%
Means	21.10	22.37	19.87	17.40	20.91	13.28
SEM	0.69	0.79	0.74	0.51	0.99	0.83

III.VI. *Colour stability*

The longer the meat is exposed to air, the darker the meat becomes, even if stored at low temperatures. Oxygen consumption rate (OCR) and metmyoglobin reductase activity (MRA) are biochemical factors for determining the colour stability of meat (Beggan, Allen & Butler, 2004). Ledward (1985) proposed that a high MRA was the most important factor in preventing meat discolouration. We used MTG to create a preservative bio-pro-film from a catalyst

agent. Our results suggested that MTG catalyzes meat proteins, especially when mixed with extracted proteins before addition to meat steaks, and protects meats when frozen. Figure 5 shows the values for lightness, redness and yellowness of the surface in treated samples. Compared to the fresh samples, non-wrapped portions had insignificant changes in the three colour components (*L*, *a**, & *b**). Values for *a** and *b** fro non-wrapped portions were slightly higher than in fresh samples, which may be caused by condensation of the pigments on the surface while moisture was released. Otherwise, all samples showed stability in *a** and *b** values, with small reductions in *a** values. *L* values in samples treated with actomyosin, MTG, 10% MTG-actomyosin, or 30% MTG-actomyosin, were higher in fresh samples, possibly because the MTG solution contains water, which turns meat grey as the concentration of pigments is reduced, and oxygen molecules react with myoglobin. This may have had an impact on the lightness values. Figure 6 shows the values of *L*, *a** and *b** determined for the inner part of beef samples frozen at -35^{o}C and treated with different wrapping materials. Interestingly, samples treated with MTG showed a slight increase in *L* values compared to other samples, including fresh cuts. However, the ideal results for consumers is an increase in lightness and redness for frozen samples, and this of great importance in the meat industry, especially for meats stored for long times. In summary, the colour stability of tested samples suggested that MTG had no negative impact on the quality of beef steaks. The findings of this study extend the hypothesis that frozen meat steaks can be preserved by a bio-pro-film generated from the bio-catalyst agent MTG. Qualities regarded as crucial elements in meat industry, including texture, weight loss after freezing, protein quality, and colour stability were preserved.

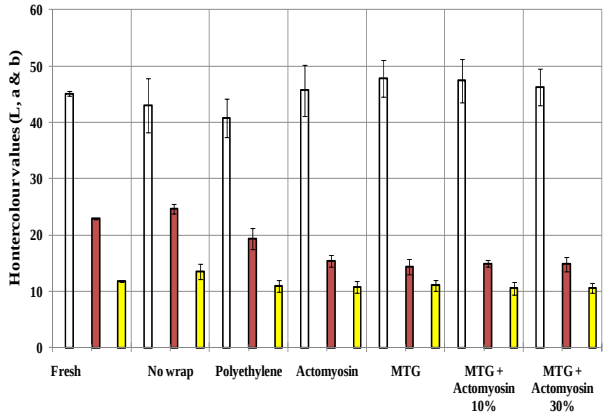

Fig. 5. Changes in colour component values (*L*, *a** & *b**) on the surface of beef steaks treated with different wrapping materials and frozen at -35ºC for 35 days.

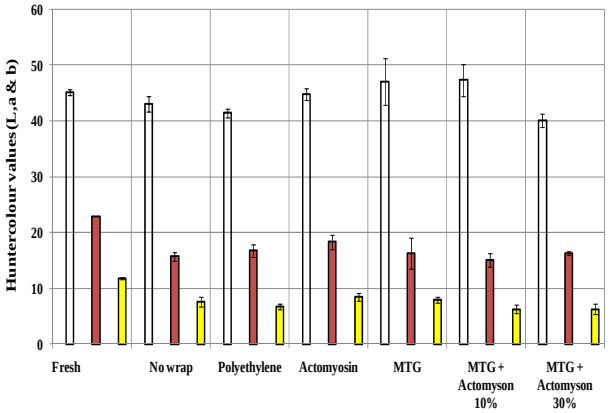

Fig. 6. Changes in colour component values (*L*, *a** & *b**) from the inner part of beef steaks treated with different wrapping materials and frozen at -35ºC for 35 days.

IV. CONCLUSION

The data showed that the ability of MTG to catalyze the crosslinking of muscle proteins is not dependent on temperature. The hypothesis that MTG could react with meat protein only at warm temperatures should be altered. The findings of this work indicated that MTG possessed the ability to react at low temperatures, and even at -35^{o}C. This alters the previous model of MTG reacting only at high temperatures. We hypothesize that the quality characterisations of the final products of frozen beef, and beef wrapped with solutions made from MTG will not differ from the original samples. We found a stability in colour, and achieved an improvement in weight loss and textural properties. From those results, we consider that MTG could be important in food preservation, if its functional properties are properly implemented to make bio-pro-films.

REFERENCES

Ahhmed, M. A. Nasu, T., Muguruma, M. (2009). Impact of transglutaminase on the textural, physicochemical, and structural properties of chicken skeletal, smooth, and cardiac muscles. *Meat Science*, 83, 759-767.

Ahhmed, M. A., Kuroda, R., Kawahara, S., Ohta, K. Koji, N., Toki, T., & Muguruma, M. (2009). Dependence of microbial transglutaminase on meat type in myofibrillar proteins cross-linking. *Food Chemistry*, 112, 354-361.

Beggan, M., Allen, P., & Butler, F. (2004). Shelf-life of retail beef muscles following storage in a low oxygen environment. *Journal of Muscle Foods*, 15, 269–285.

Ledward, D.A. (1985). Post-slaughter influences on the formation of metmyoglobin in beef muscles. *Meat Science*, 15, 149–171.

Pietrasik, Z., and Janz, M. (2009). Influence of freezing and thawing on the hydration characteristics, quality, and consumer acceptance of whole muscle beef injected with solutions of salt and phosphate. *Meat Science*, 81, 523-532.