# CHANGES IN THE HEAT-INDUCED GEL STRUCTURE AND PHYSICAL PROPERTIES OF MYOFIBRILLAR PROTEIN FERMENTED WITH PSYCHROTROPHIC LACTIC ACID BACTERIA

T. Hayashi[1]* and S. Haga[1]

[1]Department of Applied Biological Chemistry, Faculty of Agriculture,

Meijo University, Nagoya, Japan

**Abstract–The aim of this study was to investigate the gel-forming ability of muscle protein fermented with lactic acid bacteria at cold temperatures. We prepared and used myofibrillar protein, which has a key role in regard to its water-holding and binding properties in meat products, as a gelation model system. The protein was fermented with two strains of psychrotrophic lactic acid bacteria, *Lactobacillus sakei* D-1001 (commercial strain) and *L. sakei* No. 4 (in-house strain: not fully identified) at 4°C. After heat treatment at 70°C for 30 min, the physical properties and microstructure of the resulting gels were analyzed. Although fermentation profiles between the two strains were not always identical, both myofibrillar protein gels fermented with these lactic acid bacteria showed higher gel strength and syneresis rate than non-fermented gels. However, there was no distinct difference in protein distribution between the fermented and non-fermented gels. The fermented gels appeared to have a more fine and strand-like microstructure compared with the non-fermented gels. This was strongly supported by the results of image analysis of the microstructure of the gels.**

**Key Words – Muscle protein gel, Cold fermentation, Gel strength and its structure**

## I. INTRODUCTION

Despite their high protein nutritional value, it has been reported that meat and meat products are not good for health, because meat is rich in fat and cholesterol [1]. Simultaneously, focus on safe and healthy foods, such as fermented milk products, has increased in recent years.

We have tried to develop meat products with a greater added value by means of traditional food processing methods such as lactic acid fermentation and found that the physiological functions of pork meat were improved by lactic acid fermentation [2]. In addition, the color and physical properties of meat were markedly improved by lactic fermentation at moderate temperatures for most bacterial growth [3,4]. Most lactic acid bacteria have an optimum temperature of approximately 30°C; however, such temperatures are also generally considered suitable for the growth of bacteria that cause food-borne diseases. Therefore, in recent years, we have tried to apply cold fermentation to improve the food safety and quality of meat products by using psychrotrophic lactic acid bacteria, nearly identified as *Lactobacillus sakei* [5-7]. As a part of result, it was observed that myofibril fragmentation in cured meat was increased during fermentation with psychrotrophic lactic acid bacteria, even at cold temperatures [6]. From changes in myofibril fragmentation and decrease in pH values, we found that the point of inflection was located at approximately pH 5.3. Possibly, this may have a marked influence on the functional and gelling properties of myofibrillar protein, which plays a key role in the water-holding and binding properties of meat products [8]. However, very few reports have focused on the effect of lactic acid fermentation at cold temperatures on the gelation of myofibrillar protein.

In the present study, we investigated the gel-forming ability of myofibrillar protein fermented with lactic acid bacteria at cold temperatures, by measuring its physical properties and microstructure.

## II. MATERIALS AND METHODS

### *A. Sample preparation*

Myofibrils were prepared from fresh commercial pork loin, according to a procedure slightly modified from that described by Perry *et al*. [9] and Etlinger *et al*. [10]. The prepared

myofibrils were then dialyzed in 0.6 M NaCl containing 2% glucose and diluted to 15 mg/ml protein concentration using the same solution. The resultant myofibril solution was divided into three portions. The first was inoculated with *L. sakei* D-1001 (*Lb*. D-1001; commercial strain) and the second with *L. sakei* No. 4 (*Lb*. No. 4; not fully identified; our in-house strain), both at approximately $10^6$ log CFU/ml. These were selected from 16 psychrotrophic lactic acid bacteria strains that we possess [5]. The inoculated samples were fermented at 4°C until the pH reached to approximately 5.3. Thereafter, they were heated at 70°C for 30 min to form heat-induced gels. Chronological sampling was performed during fermentation to determine the pH and viable counts of bacteria. As a control, the remaining non-inoculated portion of the myofibril solution was stored at 4°C overnight and then heated at 70°C for 30 min. After heating, all samples were cooled under iced water for 30 min.

Protein concentrations were determined by biuret method (at 550 nm).

*B. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)*

Protein distribution in the fermented and non-fermented gels was analyzed by SDS–PAGE [11] on a gradient slab gel (7.5%–20%). The gels were stained with Coomassie Brilliant Blue R-250.

*C. Gel strength, syneresis rate, and protein solubility*

Comparison between the fermented and non-fermented heat-induced gels was performed according to the procedure of Fujita *et al*. [12]. A creep meter (RE2-33005S; Yamaden, Japan) was used to measure the strength of the gels, i.e., modulus of elasticity and breaking energy. These data were expressed in $N/m^3$ and $J/m^3$, respectively. Syneresis rate and protein solubility were also determined by the procedure of Fujita *et al*. [12]. The syneresis rate was expressed as the percentage weight of water released from the gel after centrifugation (580× *g*, 15 min, 4°C) over the initial weight of the gel. Protein solubility was calculated as the percentage of protein content of the gel supernatant obtained by centrifugation (35,000× *g*, 15 min, 4°C) compared with the total protein content.

*D. Scanning electron microscopy (SEM)*

The microstructure of the gels was observed using scanning electron microscope (S-3000H; Hitachi, Japan) according to the method of Haga *et al*. [13]. In brief, the gel sections (3 × 3 × 2 mm) were fixed with 2.5% glutaraldehyde, washed with 0.1 M phosphate buffer (pH 7.0), and post-fixed with 1% Osmium tetroxide ($OsO_4$). The fixed samples were then washed thoroughly with 0.1 M phosphate buffer (pH 7.0) and dehydrated in graded series of ethanol solutions (50%–100%). Each specimen was dried using a carbon dioxide critical point dryer (HCP-2; Hitachi, Japan). The dried specimens were then coated with approximately 300 Å of gold using an ion-sputtering apparatus (E-101, Hitachi, Japan). Subsequently, the specimens were observed under scanning electron microscope at an accelerating voltage of 25 kV. To analyze the gel microstructure objectively, image analysis was performed using the method of Haga *et al*. [14].

## III. RESULTS AND DISCUSSION

We used myofibrillar protein that plays an important role in the functional properties of meat products as a gelation model system [8]. Myofibrillar protein prepared from fresh pork loin was fermented with two strains of psychrotrophic lactic acid bacteria, *Lb.* D-1001 and *Lb.* No. 4, at 4°C. Viable counts of the bacteria in both inoculated samples were maintained at $10^7$–$10^8$ CFU/ml when the pH reached approximately 5.3. The samples inoculated with *Lb*. No. 4 and *Lb*. D-1001 required approximately 14 h and 17 h, respectively, till the end of fermentation (data not shown). The effect of cold fermentation on the physical properties of the heat-induced myofibrillar protein gels is summarized in Table 1. Both myofibrillar protein gels fermented with these bacteria showed significantly higher breaking energy and modulus of elasticity compared with the non-fermented gel ($P < 0.05$). The syneresis rates of the fermented gels were also significantly higher than that of the non-fermented gel ($P < 0.05$). In contrast, protein solubility was decreased in both fermented gels. These results suggest that relatively greater amounts of protein were involved in heat-induced gel formation, corresponding to reinforcement of the gel strength caused by lactic acid fermentation [15].

Table 1 Physical properties of the heat-induced gels with or without cold lactic acid fermentation

| | Non-fermentation | *Lb*. D-1001 | *Lb*. No. 4 |
|---|---|---|---|
| Breaking energy ($J/m^3$) | 316.90 ± 206.40 a | 2352.4 ±1803.9 b | 2068.3 ±1648.7 b |
| Modulus of elasticity ($N/m^2$) | 104.20 ± 56.60 a | 1145.6 ±565.60 b | 1013.5 ±500.00 c |
| Syneresis rate (%) | 73.29 ± 1.62 a | 76.69 ±2.19 b | 75.88 ±1.43 b |
| Protein solubility (%) | 6.60 ± 0.04 a | 2.62 ±0.42 b | 3.06 ±0.28 b |

Values in a row with the same letters are not significantly different ($P > 0.05$).

SDS–PAGE was performed to determine if there were differences in protein composition between the fermented and non-fermented myofibrillar protein gels. Fig.1 shows that major myofibrillar protein bands, myosin heavy chain, actin, and α-actinin were clearly observed in both fermented and non-fermented gels, and there were no distinct differences in the protein components of the gels.

As shown in Fig. 2, the fermented gels appeared to have a more fine and strand-like microstructure compared with the non-fermented gel. This subjective observation was strongly supported by the results of image analysis of the microstructure of the gels, although distinct differences between *Lb*. D-1001 and *Lb*. No. 4 inoculated samples were not detected (data not shown). In contrast, the results of differential scanning calorimetry analysis indicated that lactic acid fermentation had a strong effect on the thermal stability of myofibrillar protein, i.e., denaturation transition temperature and denaturation enthalpy of myofibrillar protein were decreased with fermentation (data not shown). Totosaus *et al*. [16] explained that the gelation phenomenon requires a driving force to unfold the native protein structure, followed by aggregation retaining a certain degree of order in the matrix formed by the association between protein molecules.

These results suggest that changes in the microstructure and physical properties of the heat-induced gels following lactic acid fermentation could be due to further inter- and intra- molecular

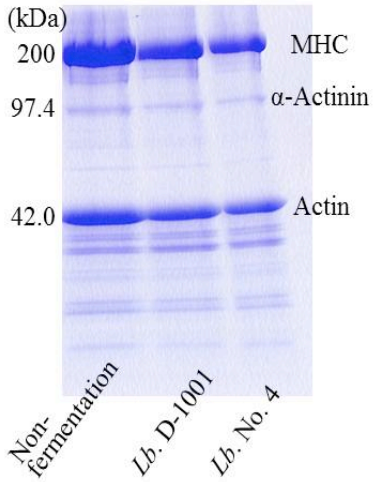


Figure 1. SDS–PAGE patterns of components in the myofibrillar protein gels with or without fermentation using psychrotrophic lactic acid bacteria

MHC: Myosin heavy chain

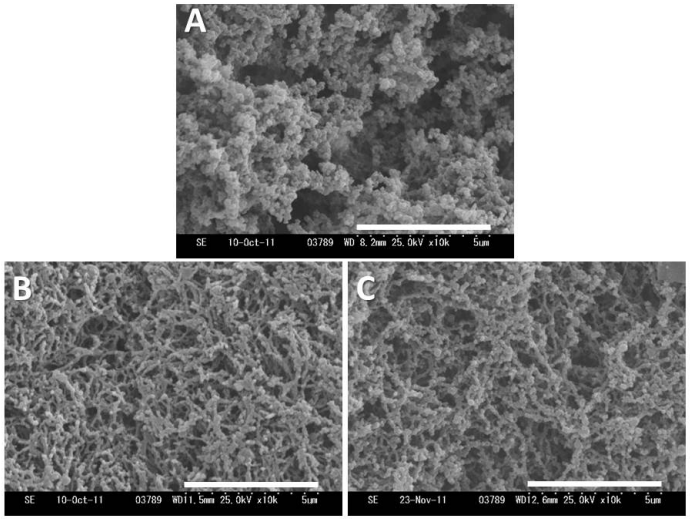


Figure 2. Comparison of microstructures of the heat-induced gels with or without cold lactic acid fermentation using psychrotrophic lactic acid bacteria

A: Non-fermented gel
B: Fermented gel (*Lb*. D1001)
C: Fermented gel (*Lb*. No. 4)
Scale bar in each image is 5 μm.

reactions caused by the fermentation, resulting in more solid gel formation.

Therefore, this cold fermentation with our *Lb*. *sakei* strains may be useful not only in regard to

food safety but also for improving the functional properties of meat products.

## IV. CONCLUSION

The results of the present study indicate that the higher physical properties of myofibrillar protein gels must be closely attributing the formation of a more fine and strand-like structure induced by the lactic acid fermentation. This fermentation at cold temperature can improve the functional properties of meat products.

## ACKNOWLEDGEMENTS

This study was supported in part by a research grant from Meat and Meat Products (The Ito Foundation), by the Academic Research Institute of Meijo University, and by a grant from the Strategic Research Foundation Grant-aided Project for Private Universities from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (S0801056).

The psychrotrophic lactic acid bacterial strains were kindly provided by San-Ei Sucrochemical Co., Ltd., Japan.

## REFERENCES

1. Li, D., Siriamornpun, S., Wahlqvist, M.L., Mann, N.J. & Sinclair, A.J. (2005). Lean meat and heart health. Asia Pac. J. Clin. Nutr. 14(2): 113-119.
2. Hayashi, T., Kato, K. & Haga, S. (2008). Angiotensin I-converting enzyme inhibitory peptide derived from porcine skeletal muscle myosin fermented by *Lactococcus lactis* subsp. *lactis* IFO-12007. J. Res. Inst. Meijo Univ. 7: 71-80.
3. Haga, S., Kato, T. & Kotsuka, K. (1994). Effects of curing and fermenting time on the quality of hams fermented with psychrotrophic lactic acid bacteria. Nippon Shokuhin Kogyo Gakkaishi 41(11): 797-802 (in Japanese).
4. Teramoto, K. & Haga, S. (2001). Effect of lactic acid fermentation on formation of myoglobin derivatives. Sci. Rep. Fac. Agr. Meijo Univ. 37: 129-134 (in Japanese).
5. Teramoto, K., Sato, H., Sun, L., Torimura, M., Tao, H., Waguri, S., Hayashi, T. & Haga, S. (2007). Rapid identification and classification of psychrotrophic lactic acid bacteria by matrix-assisted laser desorption/ionization mass spectrometry. Bunseki Kagaku 56(12): 1063-1070 (in Japanese).
6. Haga, S., Hayashi, T., Oba, M. & Sakata, R. (2009). Muscle protein degradation in pork meat fermented by psychrotrophic lactic acid bacteria. J. Res. Inst. Meijo Univ. 8: 91-96 (in Japanese).
7. Hayashi, T., Washio, S., Arakawa, M., Taguchi, M., Toyoda, N. & Haga, S. (2011). Evaluation of the functional properties of pork meat fermented with psychrotrophic lactic acid bacteria. In Proceedings 57th International Congress of Meat Science and Technology (p. 291), 7-12 August 2011, Ghent, Belgium.
8. Asghar, A., Samejima, K. & Yasui, T. (1985). Functionality of muscle proteins in gelation mechanisms of structured meat products. CRC Crit. Rev. Food Sci. Nutr. 22: 27-106.
9. Perry, S.V. & Grey, T.C. (1956). A study of the effects of substrate concentration and certain relaxing factors on the magnesium-activated myofibrillar adenosine triphosphatase. Biochem. J. 64: 184-192.
10. Etlinger, J.D. Zax, R. & Fishman, D.A. (1976). Compositional studies of myofibrils from rabbit striated muscle. J. Cell Biol. 68: 123-141.
11. Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.
12. Fujita, T., Hayashi, T. & Haga, S. (2006). Research on changes of physical properties of the actomyosin gel by retorting. Nippon Shokuhin Kagaku Kogaku Kaishi 53(8), 423-429 (in Japanese).
13. Haga, S. & Ohashi, T. (1984). Heat-induced gelation of a mixture of myosin B and soybean protein. Agric. Biol. Chem. 48: 1001-1007.
14. Haga, S., Iwakiri, T. & Mizuno, T. (2001) Studies on the physical properties and the analysis of microstructure image of meat protein gel. Sci. Rep. Fac. Agr. Meijo Univ. 37: 89-94 (in Japanese).
15. Renard, D., Velde, F. van de & Visschers, R.W. (2006). The gap between food gel structure, texture and perception. Food Hydrocolloids 20: 423-431.
16. Totosaus, A., Montejano, J.G., Salazar, J.A. & Guerrero, I. (2002). A review of physical and chemical protein-gel induction. Int. J. Food Sci. Technol. 37: 589-601.