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Effect of Fermentation with Psychrotrophic Lactic Acid Bacteria on Microstructure and Physical Properties of Heat-Induced Myofibrillar Protein Gels

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In this study, muscle protein gel-formation during fermentation by lactic acid bacteria at 4℃ was investigated using myofibrillar protein as a model gelation system. Protein fermentation by the two strains of psychrotrophic lactic acid bacteria *Lactobacillus sakei* D-1001 and *Lactobacillus sakei* No. 4 was performed at 4℃ . Physical properties and microstructure of gels were examined after treatment at 70℃ for 30 min. Both myofibrillar protein gels showed higher gel strength and syneresis rate than non-fermented gels. Whereas protein distributions did not significantly differ between fermented and non-fermented gels, a finer strand-like microstructure was more apparent in fermented gels.

Key words: muscle protein gel, psychrotrophic lactic acid bacteria, cold fermentation, gel strength, microstructure, meat products

1. Introduction

In recent years, safety and healthiness of food products, including milk-based fermented animal products such as yogurt and cheese, have received much attention. However, because meat is rich in fat and cholesterol, meat and meat products are not considered healthy, despite their high protein content and excellent nutritional value [1-3]. Lactic acid fermentation prevents the growth of pathogenic bacteria and improves texture, color, and functionality of meat products [4-6]. However, most lactic acid bacteria have optimal activities at approximately 30℃. Because growth of pathogenic food-borne bacteria are also optimal at this temperature, recent studies have examined low temperature fermentation by psychrotrophic lactic acid bacteria such as *Lactobacillus sakei* to improve the food safety and quality of meat products [7,8]. In our previous study, functional properties of meat products were examined in model pork sausages that were fermented using psychrotrophic lactic acid bacteria. In these experiments, rheological properties [4], color [5], and physiological functions such as angiotensin-converting enzyme inhibitory activity were improved by lactic acid fermentation [6]. In addition, protein denaturation and myofibril fragmentation were increased by fermentation of the model sausages at 4℃ [8].

Given these observations, lactic acid fermentation may also directly influence gelling properties of myofibrillar protein, which is a major component of muscle protein. However, very few studies have specifically examined the effects of low-temperature lactic acid fermentation on myofibrillar protein gelation. Thus, in the present study, myofibrillar protein was examined as a model gelation system that plays important roles in binding, water-retention, and rheological properties of meat products [9].

In the present study, we investigated the gel-forming ability of myofibrillar protein after fermentation by lactic acid bacteria at 4℃ by measuring physical properties and microstructure.

2. Experimental

2.1. Sample preparation

Myofibrils were prepared from fresh commercial pork loin (6 months old three-way cross pork) using procedures described by Perry *et al*. [10] and Etlinger *et al*. [11] with slight modifications. Prepared myofibrils were then dialyzed in 0.6 M NaCl containing 2% glucose and were diluted to 15 mg/ml protein in the same solution. The resulting myofibril solution was divided into three portions. The first was inoculated with *Lactobacillus sakei* D-1001 (*Lb*. D-1001; commercial strain) and the second with *Lactobacillus sakei* No. 4 (*Lb*. No. 4; not fully identified; in-house strain) at approximately 10^6 log CFU/ml (3-ml aliquots in 18×80 mm Pyrex glass tubes). These strains were selected from 16 psychrotrophic lactic acid bacterial strains from our laboratory [7]. Inoculated sam-

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ples were fermented at 4℃ until the pH reached approximately 5.3. Thereafter, samples were heated at 70℃ 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, remaining non-inoculated portion of the myofibril solution (pH 6.5) was stored at 4℃ overnight and then heated at 70℃ for 30 min. After heating, all samples were cooled in iced water for 30 min, and were stored at 4℃ until use. Protein concentrations were determined spectrophotometrically using the biuret method at 550 nm.

2.2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein distribution in fermented and non-fermented gels was analyzed using SDS-PAGE [12] on a gradient slab gel (7.5%-20%). Gels were stained with Coomassie Brilliant Blue R-250, and were then scanned using a digital photo scanner (GT-X750; Seiko Epson, Japan) at a resolution of 600 DPI.

2.3. Gel strength, syneresis rate, and protein solubility

Comparison between fermented and non-fermented heat-induced gels was performed according to a procedure reported by Fujita *et al*. [13]. A rheoner (RE2-33005S; Yamaden, Japan) was used to measure moduli of elasticity and breaking energies of gels; expressed in N/m^3 and J/m^3, respectively. Syneresis rate and protein solubility were also determined using a procedure reported by Fujita *et al*. [13]. Syneresis rate was expressed as the percentage of water released from the gel after centrifugation at 580×g for 15 min at 4℃. Protein solubility was calculated as the percentage of total protein in gel supernatants that was precipitated by centrifugation at 35,000 ×g for 15 min at 4℃. Statistical analyses were performed using Student's t-test and ANOVA with Microsoft Excel.

2.4. Scanning electron microscopy

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

2.5. Differential scanning calorimetry

Thermal properties of fermented and non-fermented myofibrillar protein were measured using differential scanning calorimetry (DSC6100; Seiko Instruments, Japan). Measurements were recorded from 9℃ to 150℃ at a heating rate of 1℃/min. Samples were then introduced into a calorimetric silver pan (70 μl), and the same volume of water was used as a reference. The enthalpy of denaturation (ΔH) was determined according to a method described by Fujita *et al*. [13].

3. Results and Discussion

The effects of cold fermentation on the physical properties of heat-induced myofibrillar protein gels are summarized in Table 1. Both fermented myofibrillar protein gels had significantly higher breaking energies and moduli of elasticity compared with non-fermented gel ($P<0.05$). Syneresis rate of fermented gels was slightly but significantly higher than those of non-fermented gel ($P<0.05$). Presumably, increased syneresis rate with fermentation is correlated with the isoelectric point of myosin (approximately 5.2). In contrast, protein solubility was decreased in both fermented gels. These results suggest that increased quantities of protein were involved in

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

	Non-fermented	*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

Data are expressed as the mean±SD of four independent experiments. Values with different letters are significantly different ($P<0.05$).

heat-induced gel formation, leading to increased gel strength after lactic acid fermentation [15, 16].

SDS-PAGE was performed to compare differences in protein composition between fermented and non-fermented myofibrillar protein gels. Figure 1 shows major myofibrillar, myosin heavy chain, actin, and α-actinin protein bands in both fermented and non-fermented gels, and indicates similar protein distributions in all three lanes.

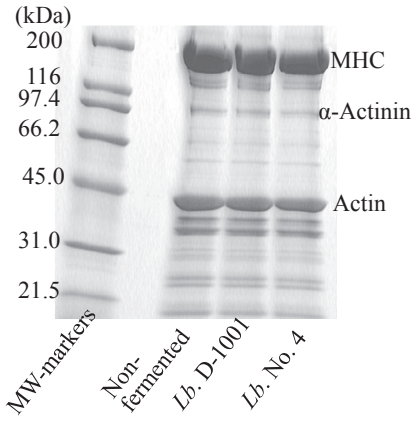

Fig. 1 SDS-PAGE patterns of myofibrillar protein gel components with or without fermentation using psychrotrophic lactic acid bacteria.
MW-markers: Molecular weight markers, MHC: Myosin heavy chain

Gel microstructure was observed using SEM. As shown in Fig. 2, fermented gels had a finer fibrous strand-like microstructure compared with non-fermented gel, which showed aggregate-like structure. In addition, scanning calorimetry analysis indicated a strong effect of lactic acid fermentation on the thermal stability of myofibrillar proteins. The denaturation enthalpy (ΔH) of myofibrillar proteins was decreased after fermentation, with values of 1.497, 0.696, and 0.934 (mJ/mg) in control, *Lb*. D-1001, and *Lb*. No.4 samples, respectively.

Fretheim *et al*. [17] reported that decreasing pH of myofibrillar protein solutions to below its isoelectric point (approximately pH 5.2) caused random aggregation and prevented formation of self-supporting gel network. In contrast, gradual reductions in pH during overnight dialysis against solutions of pH 3.5–5.5 produced sufficient protein denaturation for interactions that form a strand-like network structure [18]. In the present study, the pH of fermented samples reached 5.3 over 15–17 h. Therefore, gradual lowering of pH during low-temperature fermentation induced formation of strand-type gel network. However, lactic acid fermentation may also produce various metabolites during acidification, in part by affecting enzyme activities. Hence, further studies are required to elucidate gelation mechanism during low-

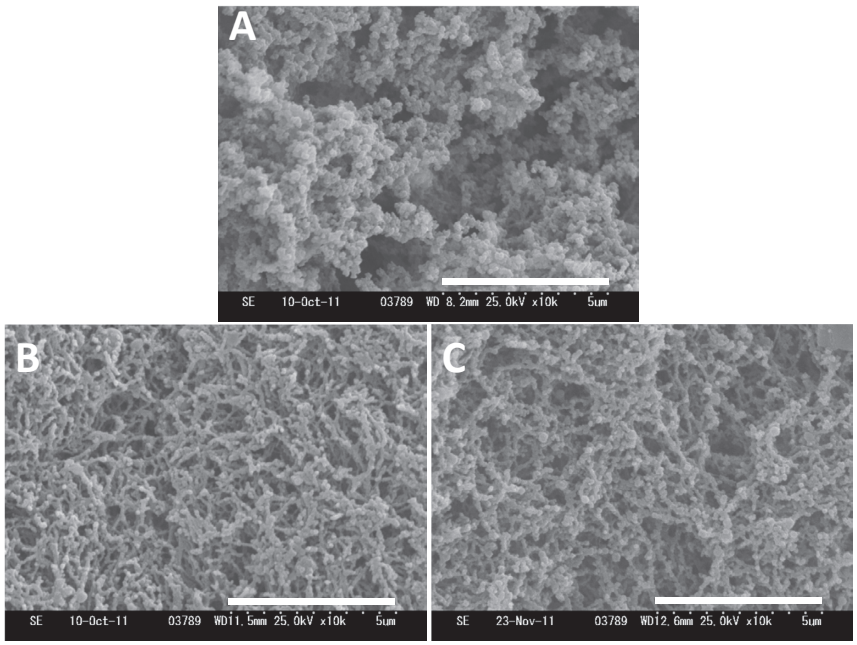

Fig. 2 Comparison of microstructure of heat-induced gels with or without cold lactic acid fermentation using psychrotrophic lactic acid bacteria.
A: Non-fermented gel, B: Fermented gel (*Lb*. D1001), C: Fermented gel (*Lb*. No. 4), Scale bar, 5 μm

temperature fermentation of myofibrillar protein.

The present data suggest that differences in microstructure and physical properties of heat-induced gels following lactic acid fermentation reflect further inter- and intra-molecular reactions caused by slow acidification, resulting in formation of more solid gels. Therefore, cold fermentation with these *Lb. sakei* strains may improve food safety and functional properties of meat products.

4. Conclusion

The present data indicate that increased physical strength of myofibrillar protein gels follows formation of a finer strand-like structure during low-temperature lactic acid fermentation.

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