Effect of high hydrostatic pressure in combination with low salt content for the improvement of texture and palatability of meat gels

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Abstract. The effect of high hydrostatic pressure (HHP) treatment of 200 MPa for 10 min at room temperature in combination with salt (sodium chloride) and phosphate (sodium pyrophosphate) addition on the microstructure and sensory characteristics of beef gels were investigated. Sensory evaluation was involving students and staff of the Niigata University, using Scheffe's paired comparison method with grade. Gel microstructure was observed using scanning electron microscopy (JSM-6510LA, JEOL LTD., Japan). The free amino acid content of beef gels was analyzed by an amino acid analyzer (JLC-500/V, JEOL LTD., Japan). The results of sensory evaluation revealed that high pressure at 200 MPa improved the cohesiveness and springiness (elasticity) while significantly decreasing the softness in low salt and/or low phosphate beef gels (p < 0.05). Irrespective of the salt and/or phosphate content in the beef samples, panellists noted that the pressurized beef gels were firmer, more elastic, and more pleasant to bite. The scanning electron microscopy images revealed that high pressure treatment at 200 MPa produced a dense, homogeneous networked structure with small cavities for water retention in low salt beef gels. The total free amino acid content of low salt and/or low phosphate beef gels was increased by high pressure at 200 MPa (p < 0.05). The present study confirmed that high hydrostatic pressure at 200 MPa was effective in producing low salt and/or low phosphate beef gels while providing high quality textural and organoleptic properties.

1. Introduction
The food industry, and in particular, the meat industry is facing global challenges such as rapid population growth, changing dietary habits and trends, and scarce resources. On the one hand, an increasing world population with a projected 10 billion people by 2050, demands sustainable and healthy nutrition. On the other hand, diseases related to nutrition, such as hypertension, obesity, and diabetes, are the cause of a large number of deaths worldwide. Various strategies, technologies, and innovative ingredients are developed and applied to ensure better availability, higher quality, and acceptability of healthier foods with reduced content of food additives. Numerous efforts are ongoing...
targeting the reduction of salt (sodium chloride), inorganic phosphate (sodium phosphate), fat, and sugar content in foods to meet recommendations of WHO. Plant-based protein ingredients (legume sources) and hydrocolloids are offered to replace fat in the meat products. Plant-based substances such as plant extracts, spices, or herbs are used to provide antimicrobial and/or antioxidant properties. Innovative natural ingredients stimulating trigeminal senses, mimicking salt-congruent flavors, or boosting salty taste are adopted to salt reduction in meat products [1]. However, there are still practical limitations of salt reduction. A weaker sensory perception of saltiness and overall flavor in the sodium-reduced products constitute, probably, the most important constrain [2].

High hydrostatic pressure technology has been recognized as a useful method for successfully reducing sodium chloride, sodium phosphate, and/or fat content [3-7]. On the other hand, this technology has been proposed as a tool to increase saltiness perception in meat products [2, 8]. The texture, yield, and organoleptic properties of processed meat products are closely related to the structure and functionality of myofibrillar proteins. Application of high pressure treatment at 100-200 MPa has been confirmed to improve the functional properties of myofibrillar proteins by modifying the structure of myofibrils due to denaturation, solubilization, aggregation, or gelation. It leads to change in the textural properties, enhancing water binding and the stability of meat gels. High pressure enhances the ability to reduce sodium, providing a high binding of meat particles and moisture retention along with the ability to change the perception of saltiness. Application of different processing conditions of high pressure could be a powerful strategy for the meat industry in producing healthy processed meat products with reduced salt, phosphate, and/or fat content.

The objective of this study was to investigate the effect of high hydrostatic pressure (HHP) treatment of 200 MPa for 10 min at 20 ± 5 °C in combination with salt (sodium chloride) (0-2%) and phosphate (sodium pyrophosphate) (0-0.5%) addition on the microstructure, sensory characteristics, and free amino acid content of beef gels.

2. Materials and methods

2.1. Materials

The meat samples were obtained from Australian frozen beef (silverside), which were purchased from the Itoham Foods Inc. Prior to using in experiments, the beef was partially thawed overnight at 4 °C. After removal of all visible connective tissue and fat, beef was sliced to an appropriate size, placed in vacuum-sealed polyethylene bags, frozen at -80 °C for 3 h, and then stored at -20 °C. All the experiments were carried out with the same frozen beef. Before each experiment, a small quantity of beef was thawed overnight at 4 °C and used for the preparation of beef gels. The pH of the muscles was from 5.55 to 5.70.

2.2. Preparation of beef gels

The beef was chopped through a 16.0-mm, then 3.2-mm plate using a mincer machine (MINCER BONNY BK-220, Japan). The chopped meat was mixed with sodium chloride (NaCl) (0-2%) and/or sodium pyrophosphate (SPP) (Na₄P₂O₇ • 10H₂O) (0-0.5%) in various concentrations of sodium chloride and sodium phosphate for each sample. The mixing process (about 60 s) was performed using a food processor (NATIONAL MK-K78-W, Japan). The final temperature of the beef gels after being mixed was 10-12 °C. The process was carried out at 4 °C. Next, the beef gels were placed in the metal cases of 40 mm in diameter and 15 mm in height and individually vacuum-sealed polyethylene bags (N-8, 75 µm thickness, 180 × 260 mm) using a degassing sealer (FCB-200 FUJI IMPULSE, Japan). After sealing, the polyethylene bags with meat samples were placed in a larger polyethylene bags, filled with water (without air bubbles) and sealed again.

2.3. High hydrostatic pressure (HHP) treatment

High hydrostatic pressure (HHP) treatment was performed in a high pressure vessel (high pressure food processor, Dr. CHEF, KOBE STEEL, LTD., Japan). The temperature of the pressure transfer
liquid (antifreeze) was kept about 20-25 °C by means of a thermostatic jacket. Each bag with meat samples was pressurized at 200 MPa for 10 min at 20 ± 5 °C. The adiabatic temperature rise induced by high pressure was about 1.5-2 °C/100 MPa. Upon the high pressure treatment, the meat samples were kept at 4 °C for further studies. Non-pressurized beef gels were expressed as the beef gels treated under 0.1 MPa as the control group.

2.4. Heating procedure
The beef gels were subjected to heating in a water bath at 80 °C for 30 min until the temperature at the center of meat sample reaches 70 °C, and cooled down with ice water to the temperature at the center -20 °C (about 15 min), before their characterization.

2.5. Sensory evaluation
Scheffe's paired comparison method was used for the sensory evaluation of meat samples. The analysis was performed by students and staff of the Niigata University (a panel of 11 to 13 healthy men and women in their twenties). Untrained panellists were asked to assess softness, juiciness, no residual taste, easy to swallow, cohesiveness, moistness, springiness (elasticity), pleasant odor, and pleasant taste of thermal beef gels.

2.6. Observation by scanning electron microscope
Microstructures of beef gels were examined by scanning electron microscope (SEM). The thermal meat samples were cut to an appropriate size and pre-fixed in 0.1M glutaraldehyde solution for 1 week. After that, meat samples were shaped into a size of 5 × 5 × 20 mm, and fixed in 0.1M glutaraldehyde solution for 1 day, in 0.1 M phosphate buffer (pH 7.4) for 2 h and treated with the 2% tannic acid solution for overnight. Dehydration was carried out for 30-60 min each in an increasing series of ethanol: water ratios (60%, 85%, 90%, 95%, 100%). The samples were finally transferred into tertiary butyl alcohol, freeze-dried (VD-800F, TAITEC CO., LTD., Japan), and coated with gold by ion-coater (IB-3, EIKO CO., LTD., Japan). Observation of gel microstructure of beef samples was carried out by scanning electron microscope (JSM-6510LA, JEOL LTD., Japan) with an accelerating voltage of 12 kV and a magnification of 100-20000. The observation was done with SS30.

2.7. Measurement of free amino acid content
After heat treatments, 8 g of each meat sample was homogenized in 2.5 vol. (v/w) of distilled water for 60 s. The suspension was centrifuged at 15,000 × g for 15 min, and the supernatant was collected through filter paper. The soluble protein in the supernatant was sedimented by the addition of trichloroacetic acid and was once again centrifuged at 15,000 × g for 15 min. The resultant filtrate, which was clarified through a membrane filter (0.45-μm pore size), was analyzed with an amino acid analyzer (JLC-500/V, JEOL LTD., Japan).

2.8. Statistical analysis
The statistical significance of differences among means was evaluated using Student's t-test, and Friedman's test (Microsoft Excel).

3. Results and discussions

3.1. Effect of high pressure treatment on the sensory evaluation of beef gels
Sensory characteristics of beef gels containing various combinations of sodium chloride and/or sodium phosphate content and treated by high pressure at 0.1-200 MPa were analyzed. The most significant results of sensory evaluation for this study were selected and presented in figure 1. As shown in figure 1, the sensory characteristics of beef gels were improved by high pressure treatment at 200 MPa, irrespective of the salt and/or phosphate content. The pressurized beef gels received high scores for the 'Juiciness', 'No residual tasty', 'Easy to swallow', 'Cohesiveness', 'Moistness', 'Springiness', 'Pleasant
tasty' items compared to unpressurized beef gels. The panellists reported that the texture of pressurized beef gels was firmer, more elastic, and more pleasant to bite. The sensory evaluation of beef gels with reduced sodium chloride and/or sodium phosphate content showed that sensory parameters such as 'Cohesiveness' and 'Springiness' items were increased, while 'Softness' item was decreased by high pressure treatment at 200 MPa (p < 0.05). These data were consistent with data obtained in a previous study and showed that high pressure treatment had a significant effect on the texture of low salt and/or low phosphate beef gels by increasing the perception of hardness, cohesiveness, and springiness (elasticity) [5].

Figure 1. Sensory evaluations of beef gels containing various concentration of NaCl/SPP addition and followed by high pressure treatment at 200 MPa (versus beef gels containing various concentration of NaCl/SPP addition, high pressure treatment 0.1 MPa). Values are expressed as mean, n = 12-13 (*p < 0.05, **p < 0.01, ***p < 0.001, ~not significant).

3.2. Effect of high pressure treatment on the microstructure of beef gels
The three-dimensional network structure of a gel is an important determinant of its functional properties, such as water-holding capacity, gel strength, and texture parameters. Our previous study showed that water holding capacity, gel strength, and texture characteristics of low salt and/or low phosphate beef gels could be improved by high pressure treatment at 150-200 MPa. In the present study, the microstructure of beef gels treated by high pressure at 200 MPa contained many solubilized filaments that formed a dense, filamentous network structure with small cavities for water retention. As shown in figure 2, in comparison to control beef gel containing 2% NaCl + 0.5% SPP addition, an overlapping filamentous network structure with prevailing small cavities was obtained in low salt and/or low phosphate beef gels by high pressure treatment at 200 MPa.
3.3 Effect of high pressure treatment on the free amino acid content of beef gels

High pressure treatment at 200 MPa affected the total free amino acid content (Asp, Thr, Ser, Glu, Gln, Gly, Ala, Val, Met, Ile, Leu, Trp, Phe, His, Lys, Arg) in beef gels containing various concentration of sodium chloride and/or sodium phosphate addition. The free amino acid content of low salt and low phosphate pressurized beef gels increased compared to non-pressurized beef gels (p < 0.05) (data not shown). This result suggested that combined use of low salt concentration and high pressure at 200 MPa could maintain the palatability of meat samples at the required level.

4. Conclusions

These results were consistent with the results of physicochemical parameters in our previous study and indicated that high pressure treatment at 200 MPa for 10 min combined with the addition of a low salt level was effective in producing low salt and/or phosphate thermal beef gels, having high quality textural and sensory attributes.

The poor texture of low salt and/or low phosphate meat samples can be improved by high pressure treatment at 150–200 MPa, providing an increase in cohesiveness, elasticity, and gel strength. The rearrangement of the gel structure caused by high pressure treatment was responsible for increasing the perception of hardness, cohesiveness, and springiness in pressurized beef samples. Thus, the high hydrostatic pressure technology can be used to compensate for the reduction of sodium chloride and/or sodium phosphate levels while improving textural characteristics and maintaining palatability at the required level for the production healthier, emulsified meat products with a low content of food additives.

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