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Research Article

Technological Developments for Producing Soft Meat that Retains its Original Shape and can be Preserved at Room Temperature

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Abstract

Aim: The aim of this study was to develop a technique for producing a texture-modified food that retains its original shape. In addition, we focused on the possibility of distribution and long-term preservation at room temperature.

Materials and Methods: A beef round was prepared for this experiment. Enzyme-injected samples were subjected to immersion, preheating and retortion treatments, and the effects of each treatment on the water-holding capacity and physical properties were observed.

Results: The preheating treatment affected the water-holding capacity and hardness of the preheated samples decreased. The immersion treatment positively affected water-holding capacity; however, hardness was not significantly altered.

Conclusion: The preheating treatment was effective in preventing the development of hardness from retort heating.

Keywords: *Soft Meat; Hardness; Water-Holding Capacity; Enzyme; Retort*

Introduction

Japan's aging rate (the ratio of the population aged 65 and older to the total population), which is the highest in the world [1], increased to 26.7% in 2015 [2], and is continuing to increase rapidly. One of the problems associated with the aging population is an increase in social security costs. Social security benefits were approximately 114 trillion in 2015 [3], which is approximately 2.5 times higher than that in 1990. This increase in social benefit costs leads to a chronic lack of financial resources. Sector-wise, medical costs and welfare account for 50% of the entire social benefit expenditure, and therefore, it is important to focus on providing healthy life expectancy and preventive long-term care. Malnutrition is one of the many health problems affecting elderly people.

Malnourished elderly people include not only hospital in-patients and people staying at care facilities, but also home-care patients, which is presently a significant social issue. The state of malnutrition in elderly affects physical functions, cure rate, crisis rate of complications, number of hospitalization days, and mortality rate, all of which significantly affect their life prognosis [4-7]. Therefore, management of malnutrition, which affects medical and healthcare expenses, is the responsibility of the entire society. Malnutrition can be caused by several factors, including social factors (e.g. poverty and solitude), psychological and factors (e.g. dementia and depression), diseases (e.g. organ failure and medicinal effects), dysgeusia, and anorexia due to aging [8]. Particularly, the factor which is being studied in recent years is difficulty in ingestion/swallowing. When elderly people are malnourished, their ability to ingest/swallow tends to decrease because of disuse-associated muscle atrophy and decline in the activities of daily living (ADL) [9,10]. According to the study conducted by National Centre for Geriatrics and Gerontology, about 70% elderly people took pleasure in having meals, which was prominent in the group

consisting of individuals with high body mass index (BMI) and in good nutritional status [11]. This shows that increasing the pleasure of eating might be useful for preventing malnutrition. However, when meals were provided to elderly people with decreased swallowing function, modifying the food texture (e.g. by mixing in a blender or by solidifying with a gelling agent), facilitated eating. However, most food items in such meals lost their original shape and colour, and it is hard to say that they stimulate the appetite.

To circumvent this issue, texture-modified food items that retain their original shape have been introduced in the market in recent years. However, these products are mostly distributed in refrigerated or frozen conditions. Since the Great East Japan Earthquake, concern for emergency food for patients with difficulty in ingestion has been on the rise. Consequently, the requirement for developing texture-modified food products that can be distributed and preserved for longer durations at room temperature is increasing.

In this study, we aimed to develop a technique for producing texture-modified food that retains its original shape and can be preserved at room temperature.

Materials and Methods

Sample preparation

We used beef rounds in this study. Beef rounds were processed into block shapes (5 cm in width, 8 cm in length, and 1.5 cm in thickness), and stored by freezing at -20˚C. The frozen beefs were thawed in a refrigerator (4˚C) overnight, and were used under different conditions.

The samples were subjected to an immersion treatment. The immersion treatment included the use of alkaline solution (0.1 mol/l sodium bicarbonate), acid solution (0.7 mol/l acetic acid), or salt solution (1.0% NaCl). A sample immersed in distilled water was used as the control. Each solution was poured in a beaker, and the samples were subjected to the immersion treatment at 4˚C for 24h.

The samples were removed from the immersion solutions after the incubation period and subjected to primary heating (preheating) treatment using a steam convection oven (Self Cooking Center, RATIONAL Co., Ltd, Japan). The preheating treatment was as follows: heating mode, steam; heating temperature, 60˚C or 80˚C; and humidity, 100%. The heating time was the time taken for the temperature at the centre of each sample to reach the heating condition (60˚C or 80˚C) and then maintaining that temperature for 1 min (heating time: approximately 20 minutes at 60˚C and approximately 15 minutes at 80˚C).

The samples were further treated with a commercial enzyme preparation containing protease (MeTORON, Christer Corporation Co., Ltd, Japan). The concentration of the enzyme solution was adjusted to 2.0% by mixing with water, after which it was injected into the samples using a disposable syringe (TERUMO Co., Ltd, Japan) until a 5% increase in meat weight. The samples injected with the enzyme were refrigerated at 4˚C for 24h. After the enzyme reaction, the meat samples were cut into four pieces and heated using a retort sterilizer (SR-240, TOMY SEIKO Co., Ltd, Japan) at 120˚C for 20 minutes. After retortion, the samples were put in an incubator (IJ-240, YAMATO SCIENTIFIC Co., Ltd, Japan) at 50˚C until measurements were taken.

Measurement of water-holding capacity

Water-holding capacity (WHC) was calculated from weight changes of samples. Sample weights were measured before (initial weight) and after immersion, after preheating, and before and after retortion (final weight). Percentage uptake, preheated loss, stored loss, and retorted loss were calculated as follows: uptake (%) = [(weight after immersion-initial weight)/initial weight] × 100, preheated loss (%) = [(weight after preheating-weight after immersion)/initial weight] × 100, stored loss (%) = [(weight before retortion-weight after preheating)/initial weight] × 100, retorted loss (%) = [(final weight-weight before retortion)/initial weight] × 100.

Measurement of physical properties

Each sample was measured using a CREEP METER (RE2-3305B, YAMADEN Co., Ltd, Japan) fitted with a columnar plunger (diameter: 20 mm). Measurements were conducted under compression velocity of 1 mm/s and clearance value of 67% compression of sample height. The hardness (N/m^2) of the samples was calculated from a texture curving line. Sample temperature was measured using a laser thermometer (THERMO-HUNTER PT-7LD, OPTEX Co., Ltd, Japan) was 45 ± 2˚C.

Statistical analysis

Differences of mean values (±SD) among samples with respect to weight change rate and hardness were analysed by the t-test, Tukey's test, or Dunnett's test using the SPSS Statistics 24 software (IBM Japan, Japan).

Results

Effect of a preheating treatment

The rate of the weight changes of non-enzyme-treated, non-preheated, 60˚C-preheated, and 80˚C-preheated samples are shown in figure 1. Preheating-associated loss of the 60˚C-preheated sample was significantly lower than that of the 80˚C-preheated sample. Stored loss of the 60˚C- preheated sample was significantly higher than that of the non-preheated and 80˚C-preheated sample. Retortion-associated loss was in an ascending order in the 80˚C-preheated, 60˚C-preheated, and non-preheated samples, and there was a significant difference between non-preheated samples and preheated samples.

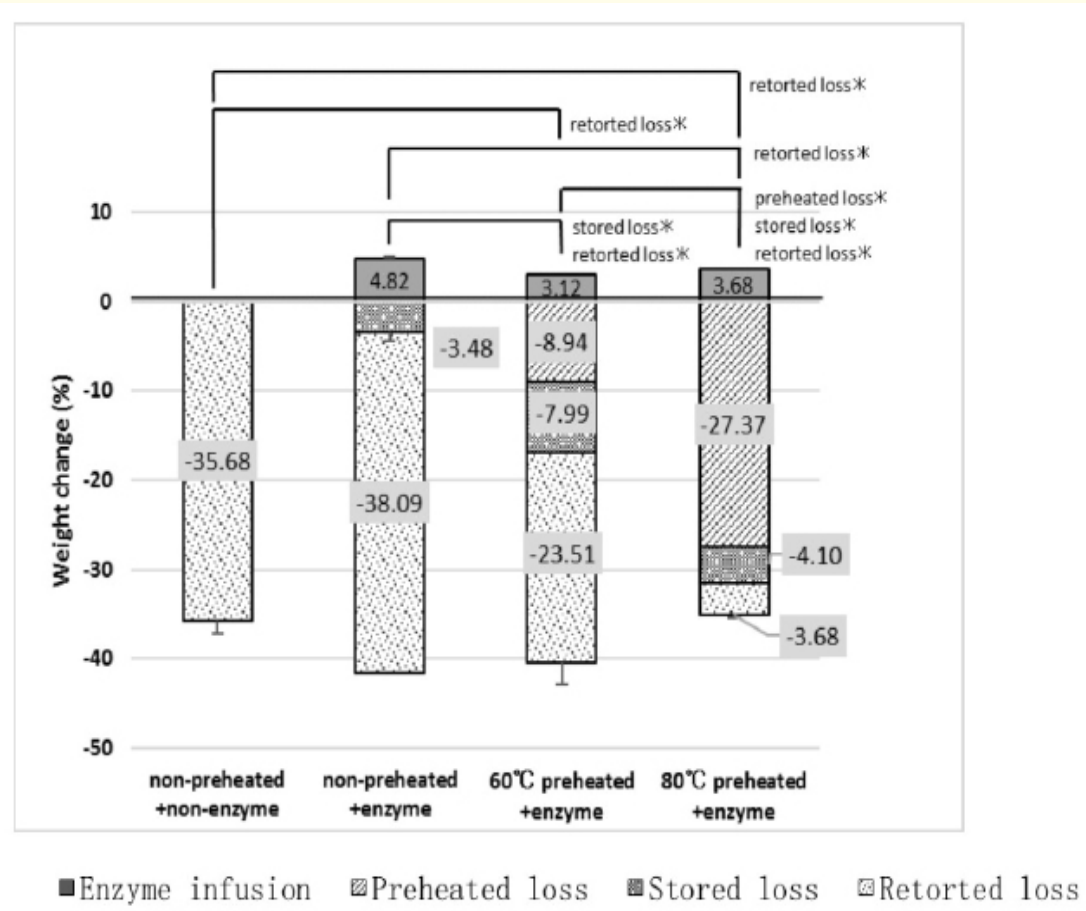

Figure 1: *Weight change of beef round samples with and without preheating treatment. Asterisks indicate significant differences between samples connected by the horizontal lines ($p < 0.05$).*

The physical properties of hardness of each sample are shown in figure 2. The hardness of enzyme-treated samples was significantly lower than that of the untreated sample. In the enzyme-treated group, the hardness was lower in both preheated samples than in the non-preheated samples. The hardness of the 60˚C-preheated sample and the 80˚C-preheated sample was almost similar.

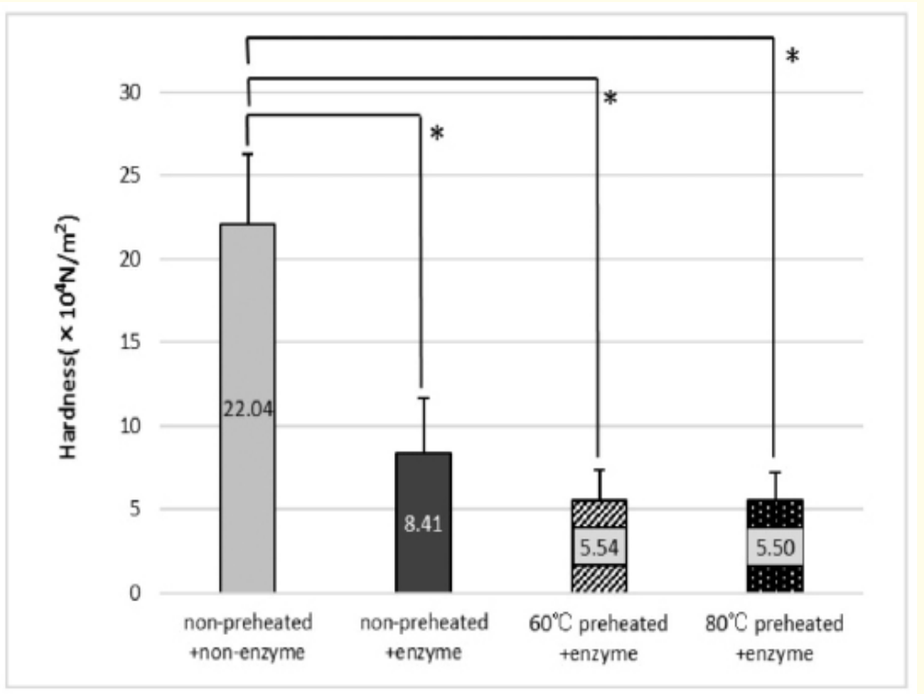

Figure 2: *Hardness of beef round samples with and without preheating treatment. Asterisks indicate significant differences between samples connected by the horizontal lines ($p < 0.05$).*

Effect of a combination of immersion and preheated treatments

The weight changes under each immersion treatment of the non-preheated, 60˚C-preheated and 80˚C-preheated samples are shown in figure 3-5. The uptakes of the immersion-treated samples were in the descending order with alkali solution, salt solution, acid solution, and distilled water. In particular, the weight changes of samples immersed in alkali solution were significantly higher than those immersed in distilled water. In the non-preheated group, the weight changes of the alkali solution sample and the salt solution sample were significantly high, and that of the acid solution sample was significantly low compared to that of the distilled water sample. The preheating-associated loss of the samples immersed in alkali and salt solutions were significantly lower than that of samples immersed in distilled water at 60˚C. At the 80˚C-preheated condition, only the alkali solution sample showed significant difference with the distilled water sample. Retorted loss was high for the alkali and salt solution samples preheated at 60˚C and for the alkali solution sample preheated at 80˚C compared to the distilled water sample.

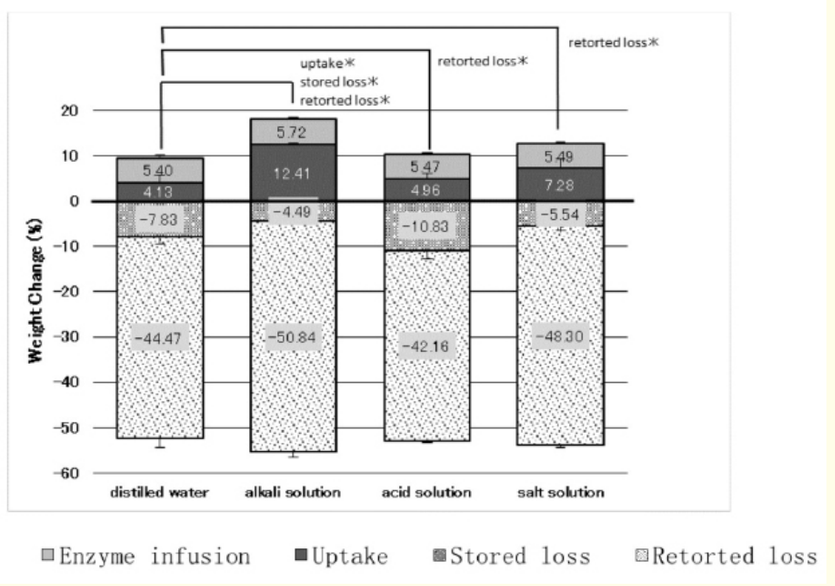

Figure 3: *Weight change of beef round samples with immersion treatment under non-preheated conditions. Asterisks indicate significant differences between samples connected by horizontal lines ($p < 0.05$).*

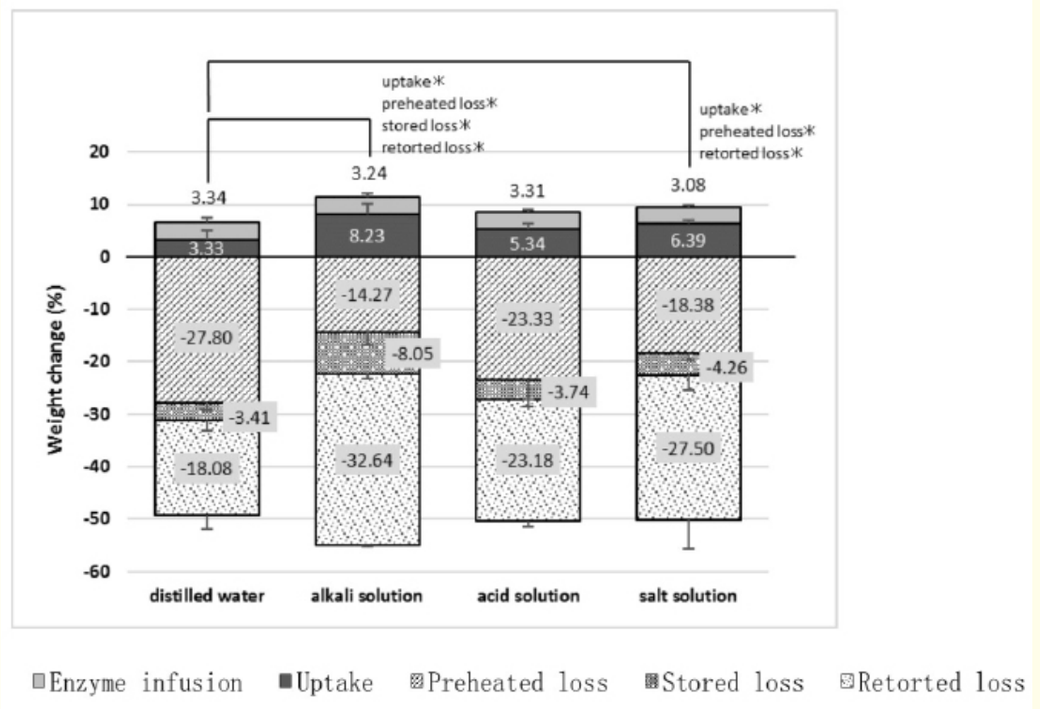

Figure 4: *Weight change of beef round samples after immersion treatment and preheating at 60°C. Asterisks indicate significant differences between samples connecting by horizontal lines ($p < 0.05$).*

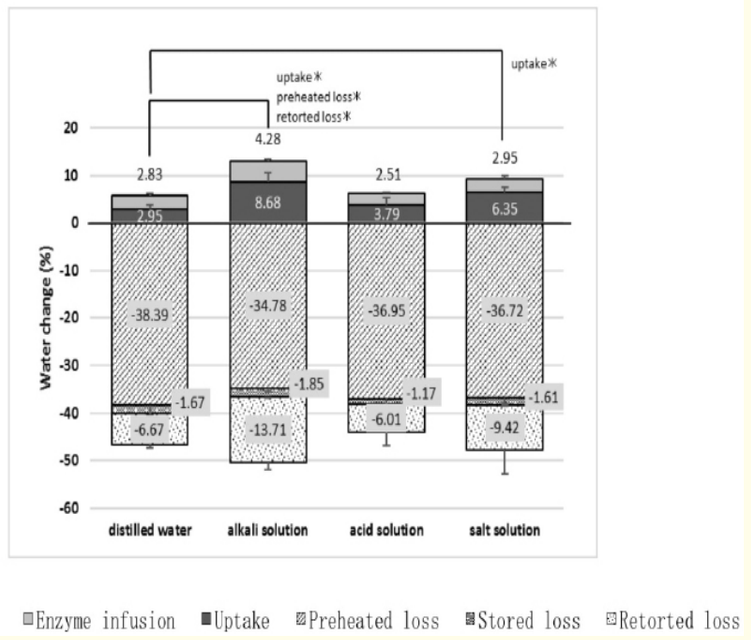

Figure 5: *Weight change of beef round samples after immersion treatment and preheating at 80°C. Asterisks indicate significant differences between samples connecting by horizontal lines ($p < 0.05$).*

The hardness value of each sample is shown in figure 6. In the non-preheated and 60°C-preheated groups, the hardness of none of the samples incubated under the three immersion conditions was significantly different from that of the distilled water-treated sample. In the 80°C-preheated group, the hardness of samples immersed in alkali solution was significantly lower than that of the distilled water-treated sample.

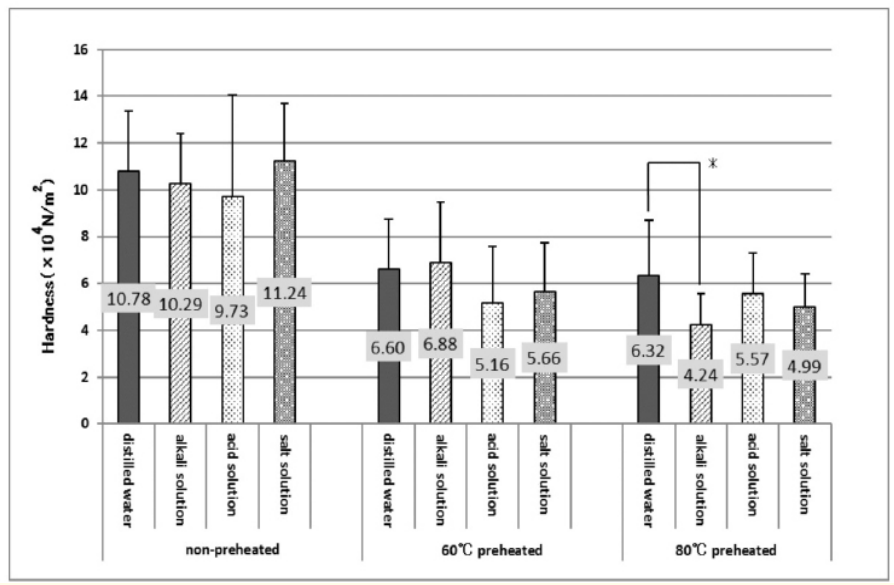

Figure 6: *Hardness of beef round samples after immersion treatment at each preheating condition. Asterisks indicate significant differences between samples connecting by horizontal lines ($p < 0.05$).*

Discussion

Effect of preheating

Japan's aging rate increased to 26.7% in 2015 [2], which is the highest in the world [1]. There are many health problems associated with elderly people, one of which is malnutrition. The state of malnutrition in elderly affects the cure rate, crisis rate of complications, mortality rate, number of hospitalization days, physical function, and life prognosis [4-7]. Therefore, management of malnutrition is an important task for the entire society. Difficulty in ingesting/swallowing food is one of the major reasons for malnutrition. Therefore, meals provided to elderly people with reduced swallowing function are texture-modified (e.g. by mixing in a blender or by solidifying with gelling agent), which eases ingestion. However, most texture-modified food products lose their original shape and colour, and it is hard to say they stimulate the appetite. Hence, in this study, we aimed to develop a technique for producing texture-modified food that retains its original shape. In addition, we determined the parameters, which will enable distribution of these food products at room temperature to enhance convenience of distribution and preservation.

The physical properties of 60˚C-preheated and 80˚C-preheated sample were lower than those of the non-preheated sample, highlighting the possibility of tenderizing food by preheating treatment. This can be explained by thermal denaturation of proteins. The primary structure of proteins, which consists of peptide-bonded amino acids, forms secondary structures such as α-helix and β-sheet, which undergo further folding to form tertiary and quaternary structures [12]. Proteins usually exist as tertiary and quaternary structures aggregated with a secondary structure, as the primary and secondary structures have weak bond energy and are easily denatured. Proteases can decrease chewiness of meat by hydrolysing the peptide bonds in protein and disrupt the fibre structure of muscle proteins. During meat tenderization, the proteases must penetrate the higher-order structure mentioned above to hydrolyse the peptide bond in the primary structure, but protein higher-order structures are stabilized by disulphide, ionic, hydrogen, and hydrophobic bonds [13,14]. However, changes in protein microenvironment by heating, for example, uncovers buried reaction groups on the protein surface, which

enables hydrolysis of peptide bonds [15,16]. Our results show that the enzyme acts more effectively and decrease chewiness of preheated samples, which may be due to such changes in protein structure. In addition, retortion heating also affects this result. The retortion heat-induced large structural changes can cause considerable shrinkage and coagulation of myofibrillar proteins at high temperature, which may contribute to development of a hard texture in non-preheated sample. On the other hand, in preheated samples, the muscle proteins had already undergone thermal denaturation, and therefore, were possibly not affected by retortion heating. This was evident from the weight changes of each sample, which indicated that the myofibrillar structure of preheated samples became relatively stronger than those of the non-preheated sample. The retortion-associated loss of the non-preheated sample was the highest, which suggested a large change in myofibrillar structure during retortion treatment. The rate of weight loss was higher with retortion time than preheating time for the 60˚C-preheated sample, whereas it was higher with preheating time than retortion time for the 80˚C-preheated sample. Thus, the difference between the two preheated samples in terms of preheating-associated loss was influenced by the preheating temperature. The thermal denaturation of proteins starts at approximately 40°C, with sarcoplasmic protein and myosin being denatured at 40 - 60˚C [17,18], collagen at 60 - 70˚C [19,20], and actin at 70 - 80˚C [18]. Cooking water loss increased with temperature in the range of 45 to 75 - 80˚C, which reaches the limit at temperatures exceeding 80˚C [14,21]. In particular, water loss increases rapidly at around 65˚C, because of cooperative shrinkage between collagen and muscular fibre [14,22]. In this study, the preheating-associated loss for the 60˚C-preheated sample was relatively low because the preheating treatment thermally denatured sarcoplasmic protein and myosin. On the other hand, denaturation of collagen and actin also occurred in the 80˚C-preheated sample and most of the heat-induced shrinkage was complete, which accounts for the higher preheating-associated loss.

Despite the difference between the two preheated samples regarding preheating-associated loss, there was no difference regarding hardness after retortion treatment. After preheating, the 60˚C- preheated sample was expected to be softer because it had high water-holding capacity, its shrinkage and coagulation were incomplete, and its hardness was lower than that of the 80˚C-preheated sample after retort; however, both samples were equally hard. Hence, we believe that the 60˚C-preheated sample was more strongly affected by retort heating than expected. As the 60˚C-preheated sample tended to have high retortion-associated loss similar to the non-preheated sample, it is estimated that the water contained in the 60˚C-preheated sample flowed out during retortion heating because the weak thermal denaturation of myofibrillar proteins caused rapid shrinkage of muscle fibres, resulting in increased hardness. From these results, we speculate that higher water content in meat leads to higher retortion-associated loss and increase in hardness. The difference in drip loss during the entire treatment between the 60˚C-preheated sample (that showed high loss at the time of retortion heating) and the 80˚C-preheated sample (that showed high loss at the time of preheating) was negligible, and both samples showed equal hardness. In addition, the time taken for the drip to flow out did not significantly influence the final hardness. Furthermore, there was no significant difference between the non-preheated and preheated samples in terms of rate of drip loss during the entire treatment. These observations suggested that the protease could easily access the peptide bonds because of thermal denaturation of muscle fibres during preheating, which was the main factor promoting tenderization in preheated samples.

Effect of a combination of immersion and preheated treatments

Water is the main ingredient of meat (approximately 75%). Approximately 85% water exists within the myofibril structure, where it is maintained by capillary force [23]. The moisture content in meat is generally maintained immediately after slaughter and decreases gradually with storage, processing, and heating. Water-holding capacity, which refers to the amount of water that was originally present in the meat or was added externally for maintenance of structure, is important for producing juicy and tender meat. The water-holding capacity in meat is affected by various factors such as pH condition. The water-holding capacity decreases at the isoelectric point (around pH 5.3) of actin and myosin, which are the main meat proteins, because inter-protein attraction reduces the gaps between myofibrils and forces the water in the meat to flow out [23,24]. However, the water-holding capacity can be improved by expanding the gaps between myofibrils at pH conditions different from the isoelectric point [25,26].

This tendency was apparent in the immersion-treated samples. The pH varied from high pH in the alkali solution and to low pH in the acid solution. The samples immersed in each solution had higher uptake and lower preheating-associated loss than the distilled water sample. In particular, the sample immersed in alkali solution showed significantly higher uptake and lower preheating-associated loss than the distilled-water sample, and it had the highest water-holding capacity among all immersion-treated samples. A similar tendency was observed for the sample immersed in acid solution, but significant differences were observed only upon comparison with the distilled-water treatment. Reports show that increasing water-holding capacity results in high density [27-30]. In other words, the pH differs from the isoelectric point of meat in acid or alkali immersion treatment. A pH paper test showed that the alkali solution was of pH 9 and the acid solution was of pH 3. The pH of the alkali solution was more distant from the isoelectric point than the acid solution, which resulted in high water-holding capacity. This also induced physical changes in protein structure and increased the hydrophilicity by increasing ionic strength [31]. The reason why significant differences were not observed with the acid solution is that the pH was closer to the isoelectric point and that the pH of meat, which was closer to the acidic zone, approached the isoelectric point after heating [28]. Additionally, the pH of the immersion solution approached the isoelectric point by buffer action of meat, because we observed that the pH of the acid solution after the immersion treatment increased to approximately pH 4. The buffer action of meat in low pH has been reported by Tezuka [32]. The sample immersed in salt solution showed a tendency similar to that observed in the alkali solution, which promoted higher uptake and lower preheating-associated loss than the distilled-water sample, and consequently, higher water-holding capacity. The effect of pH on water-holding capacity of salt solution samples was negligible unlike the acid solution and alkali solution samples, as there were no significant differences in pH between the salt solution and distilled water. Addition of salt was reported to enhance the solubility of muscle proteins, relaxing the tertiary structure after combining Na+ and Cl- with muscle proteins, and promoting coagulation of water-rich myofibrillar proteins after heating, resulting in high water-holding capacity [33-35]. In the preheated samples immersed in alkali and salt solutions, preheating-associated loss was low and retortion-associated loss was high. In other words, the retortion-associated loss tended to be opposite of that of preheating-associated loss. This showed that the drip loss induced by retortion heating increases, which results in high amounts of residual water in meat before heating. Furthermore, thermal denaturation and shrinkage of muscle protein occurred by a mechanism that was different from that of normal retortion heating, because the high water-holding capacity observed in the alkali and salt solution samples after preheating was not observed in the case of retortion heating.

The association between water-holding capacity and hardness is strong, and physically tender meat is known to improve the water-holding capacity [28,31]. The immersion treatment in this study was aimed at improving the water-holding capacity of the samples, with certain changes in weight; however, the hardness observed after incubating in individual immersion solutions was equivalent. This might be because more water flowed out owing to larger physical changes such as thermal denaturation or shrinkage of a muscle protein, which was accompanied by development of hardness, although the latter was reduced by preheating. Therefore, improving water-holding capacity could be effective in decreasing the hardness of meat by normal heating; however, retortion heating did not influence the final hardness of meat. A comparison of the non-preheated and preheated samples showed that the preheated sample was more tenderer in all immersion conditions. Collectively, our results suggest that the preheating treatment was effective in decreasing the hardness of meat.

Conclusion

In this study, we aimed to develop a technique for producing texture-modified food, which retains its original shape and can be distributed and preserved for longer durations at room temperature. Compared to non-preheated samples, retortion-associated loss and hardness were significantly lower in the pre-heated samples. Protease acted easily on peptide bonds owing to thermal denaturation of muscle fibre after preheating, which promoted tenderization of preheated samples. The immersion treatments had no effect on the hardness of retortion-treated samples. Thus, the preheating treatment was effective in producing retorted texture-modified food that retained its original shape.

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