



Quality Improvement of Frozen and Chilled Beef *biceps femoris* with the Application of Salt-bicarbonate Solution

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ABSTRACT : The effects of salt and bicarbonate solution on overall meat quality in beef *biceps femoris* muscle were investigated with the application of chilling and freezing conditions. Muscles were injected to a target of 120% of original meat weight with a solution containing 1.2 M sodium chloride, 0.25 M sodium bicarbonate and 0.1% ascorbic acid (pH 7.2). Half of the meat samples, considered as chill treatment and chill control, were stored at 4°C up to five days; while the other half, frozen treatment and frozen control, were kept in a freezer at -20°C for seven days. Compared with untreated control, treated meats had higher water holding capacity ($p < 0.05$), lower drip loss ($p < 0.05$) and lower shear force ($p < 0.07$) with higher overall acceptability ($p < 0.05$) in sensory evaluation. Morphological observations demonstrated smooth and gummy meat surface due to the solubilization of myofibrillar proteins and the distortion of connective tissue in treated raw meats; and in the case of cooked meat, treatment caused the fragmentation of myofibrils, which might be responsible for a lower shear value in salt-bicarbonate treated beef *biceps femoris* muscle. (**Key Words :** Beef *biceps femoris*, Salt, Bicarbonate, Meat Quality)

INTRODUCTION

The meat industries always try to supply meat with high quality, like good tenderness and water holding capacity, and with low cost at retail level. By conducting sensory analysis and shear-force test, it is already revealed that beef round steaks are less tender than other beef subprimals (Morgan et al., 1991). Many investigations have already been done to identify the toughest single muscle at beef round. The outside beef round (which consists mainly of *biceps femoris* muscle) showed the highest Warner-Bratzler shear force values and the lowest sensory tenderness ratings (Rhee et al., 2004). The meat processors and researchers are now trying to improve the meat quality and overall palatability of single beef round muscle by applying

different marinating techniques (Wheeler et al., 1991; Xiong and Kupski, 1999; Sheard and Tali, 2004) and different mechanical processes (Suzuki et al., 2006). At the same time, it is also important to establish the techniques for long-term preservation of meat without impairing the quality.

Many researches have already been done on the use of marinades (e.g., sodium chloride, phosphate, calcium chloride, etc.) in meat. For instance, trained sensory panelists evaluated that beef steaks and pork chops marinated with a phosphate/salt-containing solution were more juicy and tender (Vote et al., 2000). Besides, there are reports that the addition of marinade solutions in combination with sodium chloride-phosphate or sodium chloride-bicarbonate or calcium chloride-calcium lactate enhanced the eating quality and shelf-life of the meat (Lawrence et al., 2003; Sheard and Tali, 2004; Baublits et al., 2006). Sodium chloride is one of the important salts which not only improve the shelf-life and taste (or aroma) of the meat but also causes an increase in the solubility of meat protein which is responsible for higher water holding capacity in meat (Offer and Knight, 1988). Shear force of the tough meat is also found to be declined by CaCl_2 injection, but its high concentration makes the meat bitter

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(Gerelt et al., 2002). All of these ingredients (as described above) can have great contributions to the meat in regard to tenderness and overall meat quality.

Sodium bicarbonate is widely used as a food ingredient. Its addition to raw meat reduces drip loss and shear forces, and also increases the weight of saleable products due to the retention of added water at high pH (Hamm, 1960; Bouton et al., 1973; Wynveen et al., 2001). However, sodium bicarbonate is not easily applicable as a marinade in raw meat, because high pH condition poses an increased risk of bacterial growth. The addition of food preservatives to the marinade may be one of the options to preserve the meat, but consumers might not easily accept them with health aspect. The most common technique of meat preservation practiced by meat sellers is freezing or chilling. Though chill temperature can not damage the meat fiber, such a condition is unable to preserve the meat for a long time. On the other hand, freezing after marinating can be an acceptable easy method for preserving meat for a long time; but this influences meat quality attributes such as color, water holding capacity, tenderness, thawing loss and cooking loss (Honikel et al., 1986; Farouk and Swan, 1998; Farouk et al., 2003).

The injection of sodium chloride-sodium bicarbonate marinade into meat with the combination of ascorbic acid followed by freezing could be used for preparing "ready to cook" meat at home or restaurants. However, it is not clearly known how freezing affects the quality of the salt-bicarbonate treated beef *biceps femoris* muscle. Hence, the main objective of this research is to determine the quality characteristics of salt-bicarbonate treated meat in comparison to the untreated meat under the storage conditions of freezing and chilling.

MATERIALS AND METHODS

Sample collection and preparation

Beef *musculus biceps femoris* muscles (breed: Holstein; sex: female, age: 4 years, 3 day postmortem, muscle pH at the time of purchase: 5.55-5.75) were purchased from Fukuoka Meat Wholesale Market Inc. (Fukuoka, Japan) and immediately transported to the Laboratory of Chemistry and Technology of Animal Products of Kyushu University. After that the meat was kept at cool chamber of refrigerator (4°C) for about 2 h until they were used for the experiments.

The beef *biceps femoris* muscles of round part from three different carcasses were collected. Each muscle was cut into total 12 parts with same size and shape by using a meat slicing machine (Delonghi, Mod.SL360, China). After removing the fats, ligaments and tendons from each of the muscles as much as possible, they were randomly divided into four groups for each of the three replicated experiments (4×3). Treatment consisted of: 1) chilling only (CC); 2)

freezing only (FC); 3) chilling after treatment with solution (CT) and 4) freezing after treatment with solution (FT). The first two groups were considered as control. The last two groups were injected to a target of 120% of initial weight with solution containing 1.2 M sodium chloride, 0.25 M sodium bicarbonate (pH 7.2) and 0.1% ascorbic acid by using syringe. Ascorbic acid was expected to act as an antioxidant and in improvement of the meat color. Weights of the meat pieces, before and after injection, were recorded and they were wrapped with plastic film. Half of the total samples (CT and CC) were kept at cool condition at 4°C for five days for chilling and the rest half (FT and FC) were stored in freezer at -20°C for seven days for freezing. Before freezing, the samples were covered with plastic film and were held in a cold room (4°C) for 24 h to allow for equilibration of solution. Before analyzing the parameters of the frozen meat, it was thawed at 4°C in a cold room overnight. The experiments were replicated three times.

Measurements of injection gain, drip loss and salt concentration

Meat pieces were re-weighed after injection, after thawing or after 5 days of chilled preservation. After that injection gains and drip losses were measured by using the following calculations: (1) injection gain (%) = $((w_2 - w_1)/w_2) \times 100$, (2) drip loss (%) = $((w_2 - w_3)/w_2) \times 100$; weights of individual round steaks were recorded before injection (w_1), after injection (w_2) and before cooking (w_3). (3) Salt concentration was measured according to AOAC method (AOAC, 1997, method No 937.09).

Measurement of pH

For measuring the meat pH, each sample (5 g meat) was homogenized (using a Polytron homogenizer for 30 sec) with 10 ml of distilled water in a 20 ml test tube. The pH of homogenate was measured using an electrical automatic pH meter (Beckman Instruments, Inc., USA). The pHs of chilled raw meats were determined on day 1 and day 5 of preservation. The pHs of frozen meats were measured just after thawing.

Measurement of moisture

Moisture of meat samples was determined according to AOAC method (AOAC, 2000, Method No. 950.46). In case of chilled condition, moisture measurement was done on day 1 and day 5; while in case of frozen condition, it was done just after complete thawing.

Measurement of water holding capacity

Similar to the moisture measurement, water holding capacity (WHC) of chilled meat was measured on day 1 and day 5; and for frozen condition, it was measured after complete thawing. WHC was determined by the filter paper

press method. Each meat piece ($1 \times 1 \times 1.5 \text{ cm}^3$) was covered with 8 sheets of filter papers and 2 sheets of plastic board on each side, and then pressed with a 196 Newton (20 kg) load cell for 5 min. After that the water holding capacity was determined by using the following calculation:

$$\text{WHC (\%)} = [1 - \{(\text{meat weight before pressurization} - \text{meat weight after pressurization}) / (\text{meat weight before pressurization} \times \text{moisture content in gram})\}] \times 100$$

Measurement of cook loss

Each meat sample wrapped with a heat stable polyethylene bag was kept in water bath at 80°C until the internal temperature of the meat sample was reached to 75°C . After that the samples were kept in ice for about 30 min for complete cooling. Cook losses were calculated after draining the drip coming from the cooked meat. For measuring cook loss the following calculation was used: $\text{cook loss (\%)} = ((w_3 - w_4) / w_3) \times 100$; where, w_3 = meat weight before cooking and w_4 = meat weight after cooking.

Measurements of texture (hardness) for raw meat and shear force for cooked meat

Texture (hardness) for raw meat was measured by a Rheometer NRM-2002 (Fudoh Co. Ltd., Tokyo) with a conical plunger according to the procedure described by Gerelt et al. (2002). The penetration of the plunger was set parallel to the fiber direction of the meat. On the other hand, for the measurement of shear force, cooked *biceps femoris* muscles were cut, with fibers parallel to the long axis, and sheared at right angles to the fiber direction with razor blade (Cavit et al., 2004; Sheard and Tali, 2004). The machine was set at a speed of 0.5 mm/sec, and peak shear force was recorded. Six cores per individual sample were taken for the measurement.

Microbial counts

Meat samples of 10 g were removed aseptically from stored meats and homogenized with 0.85% sodium chloride solution for 1 min by stomacher. Cell counts were determined by standard pour plate technique using 10^{-3} and 10^{-4} dilutions. Duplicate plates were prepared for each dilution and after solidification of the agar ('Nissui' Standard Method Agar, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), plates were kept in an incubator at 37°C for 48 h. Microbial counts were repeated three times for each of the treatment.

Morphological observation

Morphological observations were done in triplicate for each of the sample and the best representative picture was selected for this article.

Digital camerawork : Before and after cooking, the

picture of meat surface was taken by a digital camera (Nikon corp., Tokyo, Japan).

Light microscopic observation : Raw meats treated with or without salt-bicarbonate solution were observed under light optical microscope (Nikon Inc., Tokyo, Japan) at a magnification of 100. For light microscopic observation, the samples were cut into thin sections of approximately 200 μm . The specimens were stained with hematoxylin-eosin.

Immunohistochemical analysis: Samples (about 5 mm^3) treated with or without salt-bicarbonate marinade solution were frozen with dry ice-acetone mixture and store at -80°C until they were used for histological preparation. Serial frozen sections (10 μm thickness) were obtained from the frozen tissue to detect type-I collagens by immunohistochemistry. The sections were washed with PBS containing 1% normal goat serum (Sigma, USA) anti-bovine collagen type-I polyclonal antibodies (Chemicon International, Canada), which were diluted at 1:200 and 1:400 each with PBS containing normal goat serum and 1% BSA, were used as primary antibodies. The sections were incubated with primary antibodies for 1-3 h. They were washed three times with PBS for 5 min, and then incubated for 1 h in a dark box with fluorescein isothiocyanate (FITC)-conjugated secondary antibody diluted at 1:150 with PBS. After immunostaining, the sections were observed by a confocal microscope (RCM800; Nikon Inc., Tokyo, Japan).

Scanning electron microscopic analysis : Morphological structure was studied by scanning electron microscopy (SEM) according to the procedure of Palka and Daun (1999) with slight modifications. Briefly, small pieces ($5 \times 5 \times 5 \text{ mm}^3$) from the control and treated raw meats and cooked meat were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 3 days. The pieces were divided into two groups, one was immersed in distilled water for several hours for an observation of cross section of raw and cooked muscle tissue, and another was in 8% NaOH for 7 days for an observation of *Perimysium* and *Endomysium* in raw muscle tissue. The distilled water was replaced by fresh one every after 1.5 h and the NaOH solution was also replaced every day by a fresh one and then rinsed in distilled water for 5 days at room temperature. Then the pieces were put in 1% tannic acid for 2 h, post-fixed in 1% osmiumtetroxide, 0.1 M phosphate buffer (pH 7.0) for 1 h, dehydrated in graded ethanol solutions of 50, 60, 70, 80, 90, 95% and absolute ethanol for 1 h in each solution and dried by the t-butyl alcohol freeze-drying method. The dried specimens were coated with gold and examined using a SEM (SS-550) with an accelerating voltage of 15 kV.

Sensory evaluation

The samples treated with or without marinade solution were subjected to sensory evaluation. Three samples

Table 1. Quality characteristics of chilled meats marinated with or without sodium chloride-sodium bicarbonate (pH 7.2)

Quality characteristics	CC meat	CT meat	Significance
On the first day of chilling			
pH	5.23±0.05	6.68±0.03	*
Injection gain	-	80.78±7.5	
Moisture content (%)	73.33±2.88	78.58±3.12	
Water holding capacity (%)	69.24±6.74	79.18±6.12	
After five-day chilling			
pH	5.75±0.03	7.19±0.11	*
Salt concentration (%)	0.22±0.02	1.49±0.02	*
Moisture content (%)	66.63±0.92	73.78±1.02	*
Water holding capacity (%)	60.95±9.38	76.81±2.58	*
Drip loss (%)	1.35±0.38	0.92±0.16	*
Cooking loss (%)	20.07±5.07	10.27±2.27	*
Total viable bacteria count (log ₁₀ CFU/g meat)	4.25±0.02	4.03±0.03	

* p<0.05; CC, Chill control; CT, Chill treatment; Mean±standard deviation.

Table 2. Quality characteristics of frozen meats marinated with or without sodium chloride-sodium bicarbonate (pH 7.2) after thawing

Quality characteristics	FC meat	FT meat	Significance
pH	5.99±0.02	7.02±0.05	**
Moisture content (%)	72.68±0.66	77.24±0.58	*
Water holding capacity (%)	62.23±1.23	76.27±3.97	*
Drip loss (%)	2.2±0.36	0.96±0.13	*
Cooking loss (%)	39.44±0.46	33.36±1.90	*
Total viable bacteria count (log ₁₀ CFU/g meat)	4.21±0.02	3.96±0.01	

** p<0.01; * p<0.05; FC, Frozen control; FT, Frozen treatment; Mean±standard deviation.

(3×3×1 cm³) from each of the muscle were cut into small pieces and were grilled at 160°C for 90 sec. The grilled samples were then given to panel members (n = 24) of the students and staffs of the Kyushu University who were not trained in the sensory analysis of meat. Ten characteristics *viz.* comprehensive acceptability, mouth feelings, tenderness, juiciness, flavor, sweetness, saltiness, acid taste, bitterness and over all taste were considered for sensory evaluation. Each of the characters was evaluated on the basis of seven point scale from -3 to +3 (very poor to excellent). Panelists were asked to evaluate the treated meat in comparison to its untreated counterpart. Briefly, first panelists tasted the untreated control meat, and then rinsed out their mouth with water. Then they tasted the treated meat, followed by marking the ranks of the considered points in the rating sheet.

Statistics

Means and standard deviations were calculated among samples and the t-test was done for significant differences between control and treated meats. One sample sign test was used for the sensory evaluation.

RESULTS AND DISCUSSION

General meat quality

Data presented in Table 1 and 2 show the general

quality of chilled and frozen beef *biceps femoris* marinated with sodium chloride-sodium bicarbonate solution. The pH of CT meat after chilling (Table 1) was higher than CC meat. The final pHs of treated meats were maintained above 7.0 (whereas control meats had a pH <6) irrespective of storage conditions (Tables 1 and 2). In agreement with our study, pH value higher than control was also observed in bicarbonate treated pork *biceps femoris* (Wynveen et al., 2001), sow loins (Sindelar et al., 2003a; b), cooked pork loin (Sheard and Tali, 2004) and pre- and post-chilled broiler breast meat (Sen et al., 2005). Hence, the higher pH in the treated meat may have been attributed by the sodium bicarbonate used for marinating the meat. The injection gain (Table 1) in treated meat was about 80%, which leads to high salt concentration (Table 1) in treated meat compared to control meat. The salt concentration (Table 1) of treated meat was about seven times higher than untreated one, because the treatment solution contained 1.2 M NaCl (the final concentration of salt in treated meat was 1.5%).

All the moisture related parameters (e.g., moisture content, WHC, drip loss and cooking loss) showed significantly better values for salt-bicarbonate treated chilled and frozen meat compared to control (Tables 1 and 2). Significantly higher moisture contents were found in both CT and FT meat compared to control. Moreover, salt-bicarbonate marinade significantly decreased drip loss and remarkably increased WHC of CT and FT meat compared

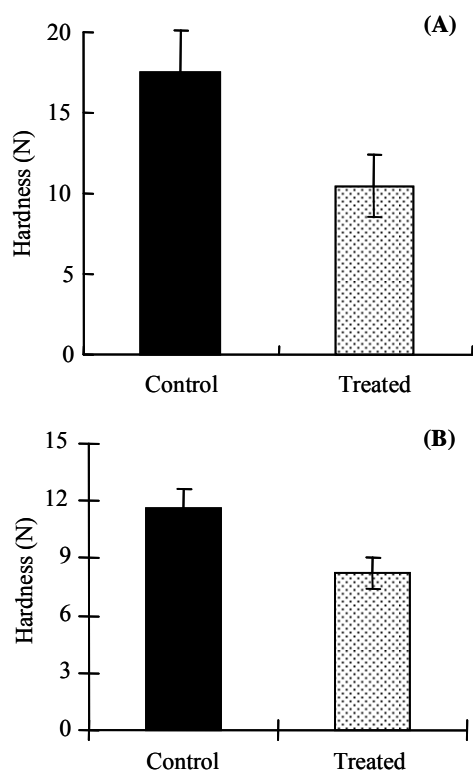


Figure 1. Changes in hardness (firmness) of salt-bicarbonate treated raw meats at different storage conditions. The detail of the texture measurement is described in the text. (A) Chilled meats 5 day after treatment. (B) Seven days frozen-thawed meats after treatment.

to CC and FC meat, respectively. An increase in WHC (about 26% for CT and 22% for FT meat) was observed in treated meat compared to control meat although a 20% of solution was injected compulsively (Tables 1 and 2). Similar to our result, Yang et al. (2006) found significantly lower drip loss and increased WHC in sodium bicarbonate treated pre-rigor porcine *longissimus lumborum* muscle. Sheard and Tali (2004) reported lower drip loss in pork loin treated with salt and bicarbonate. Sen et al. (2005) also got higher WHC in bicarbonate-salt treated broiler breast meat than phosphate treated meat and interpreted that this was due to the high buffering capacity and ionic strength of the bicarbonate-salt solution. According to the theory of Hamm (1970), the chloride ion in salt solution induces swelling in myofibrils and makes higher water retention within the protein network. Therefore, the high pH and salt concentration in meat shown in Table 1 and 2 are responsible for high retention of water in treated meat compared to control.

As widely reported (Brotsky, 1976; Babdji et al., 1982; Froning and Sackett, 1985), marinating with salt solution not only increases WHC of meat, but also reduces cook loss and increases meat juiciness. Cook losses in both CT and FT meat were significantly reduced (compared to control)

when salt-bicarbonate marinade was injected to the meat (Tables 1 and 2). In agreement with the present study, Sen et al. (2005) also reported significant reduction in cook loss of pre- and post-chilled broiler breast meat treated with bicarbonate solution. In another study, addition of NaCl at 2% level was found to reduce the cook loss of beef *biceps femoris* steaks more effectively than phosphate (Baublits et al., 2005). This was explained by the difference in the ionic strength between NaCl and phosphate used, but inexplicable, small difference compared to control meat. It is remarkable here that, compared to the CT meat, cook loss in FT meat was somewhat inconsistent (Tables 1 and 2). Beside, effect of salt-bicarbonate marinade was not sufficiently potent under the frozen condition to result in a marked difference in cook loss between control and treated meats even though WHC of the latter tended to be greater than that of former (i.e., cook loss of FC meat is 39.44% and that of the FT meat is 33.36%). The reason why the cook loss was increased in FT meat is not clear.

Texture and shear force

Analysis of texture (hardness) showed a lower penetration force in marinated meat when compared to control meat (pH<6) irrespective of chilled and frozen storages (pH>7) (Figure 1A and 1B). In accord with the present result, Ruiz-Ramírez et al. (2005) reported that a low pH ham muscle had greater hardness, cohesiveness and springiness than hams with high pH. Hamm (1986) explained that the increased hardness of meat is due to the lowering of meat pH closer to the isoelectric point of myosin which increases intermolecular linkages between positive and negative charge groups. It is suggested that less hardness (softness) of salt-bicarbonate treated meat in the present study was largely attributed to a large amount of water retained in the meat.

Contrary to the expectation, a significant difference in shear force was not observed between control and salt-bicarbonate treated cooked meats regardless of treatments (chilling or freezing), but the treated meats showed a tendency towards lower shear force than control (Figure 2A and 2B, $p<0.07$). However, with sensory analysis, higher score of tenderness was obtained for treated meat (Figure 7). The difference in objective and subjective measurements may explain part of the observed results. Stites et al. (1989) showed a significant lower shear force in beef roasts injected at 10% of their original weight with the solution containing sodium tripolyphosphate and sodium chloride. Sheard and Tali (2004) also reported that the shear force in treated pork meat (bicarbonate alone and combination of salt and bicarbonate) was less than half of the control. On the contrary, Baublits et al. (2005) reported no significant difference in shear force between phosphates treated beef and control beef meat.

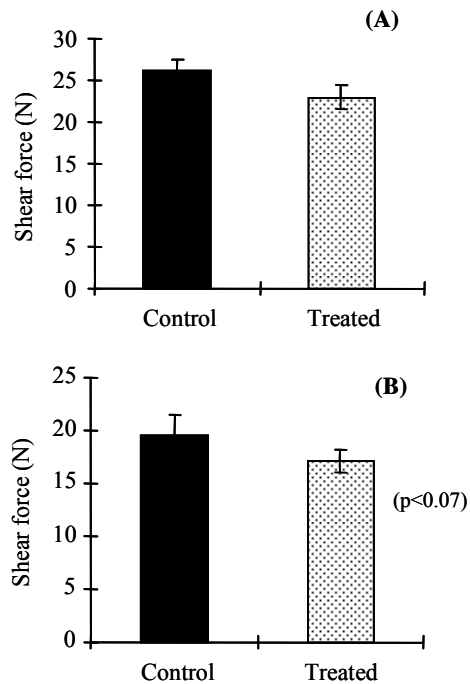


Figure 2. Effect of salt-bicarbonate treatment and storage condition on shear force of cooked meats. The details of cooking method and shear force measurements are described in the text. (A) Chilled meats 5 day after treatment. (B) Seven days frozen-thawed meats after treatment.

Microbial analysis

High pH in meat treated with the marinade in combination of sodium chloride, sodium bicarbonate and ascorbic acid was expected to allow bacteria to proliferate. However, the total viable bacteria count (TVC) in the treated meat after 5 day chilled and seven day frozen-thawed meat was within permissible limits of food hygiene (Table 1 and 2). Both CT and FT meat showed lower bacteria count than untreated control. The CT meat contained 4.03 ± 0.03 of \log_{10} CFU/g and the CC meat contained 4.25 ± 0.02 of \log_{10} CFU/g. The FT meat contained 3.96 ± 0.01 of \log_{10} CFU/g and the FC meat contained 4.21 ± 0.02 of \log_{10} CFU/g. This result was unexpected but might have been due to the effect of sodium chloride, which was much higher in the FT and CT meat. According to ICMSF (1986), the maximum limit of APC (aerobic plate count) of control raw meat is about $7 \log_{10}$ CFU/g by 8 days of chilled storage. There was a report that NaCl treated ground pork exhibited lower APC than control up to 14 days of storage at 4°C (O'Connor et al., 1993). Despite of the present result, it is to be noted that meat before freezing or after thawing always poses a risk of contamination of bacteria by handling mistakes.

Morphological analysis

Microphotographs of salt-bicarbonate treated meat

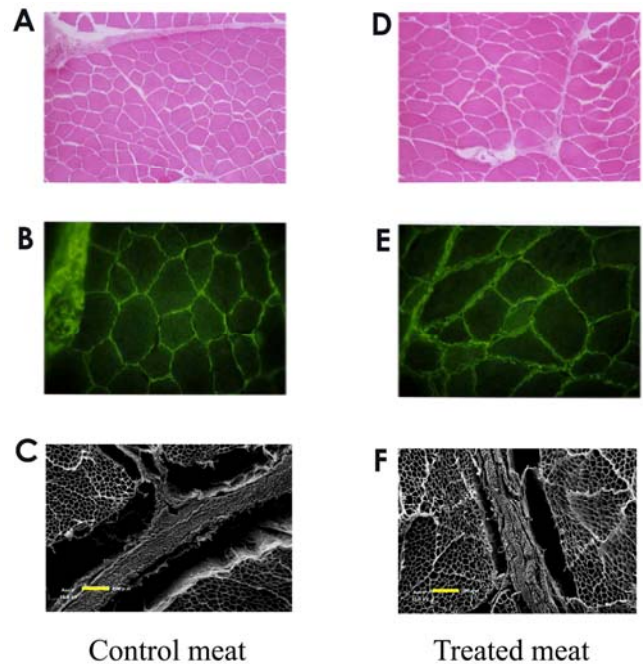


Figure 3. Morphological changes in salt-bicarbonate treated meat after 5 day chilling and 7 day freezing. (A, D) represent pictures from histochemical staining with hematoxylin-eosin; (B, E) represent immunohistochemically detected type I collagen; and (C, F) represent the SEM photographs of intramuscular connective tissue (*Endomysium* and *perimysium*). (A), (D), (B) and (E) samples were prepared from meats after 5 day chilling and (C), (F) samples were prepared from frozen meats after thawing. Each bar in (C, F) indicates 200 μm .

showed larger muscle fibers than control, which may have been attributed by swelling resulted from the compulsive injection of 20% marinade solution (Figure 3A and 3D). The dissociation of actomyosin complex by the salt-bicarbonate solution having a high pH (7.2) and salt concentration (1.2 M) would lead to an expansion of the myofibril lattices, thus allowing increased water retention. From immunohistochemical observation, the intramuscular connective tissue (Perimysium; type-I collagen) treated with sodium chloride-sodium bicarbonate solution was found clearly disordered compared to control (Figure 3B and 3E). Scanning electron microphotographs showed the endomysial honeycomb structure with empty cells after resolving out myofibers with NaOH treatment and thicker perimysia bands with slits (Figure 3C and 3F). The endomysia exhibited little differences in collagen architecture between control and treated meats. On the contrary, the perimysia took on a different aspect of collagen architecture, namely, stacks of collagen plates in control meat and loose tissue of slender collagen fibers in treated meat. These deformations shown in Figure 3E and 3F may have been caused by physical force of swelling, while there is a slight possibility that latent matrix-degrading proteinases, which plays a role in degrading the collagen in muscle, related to structural change in perimysia

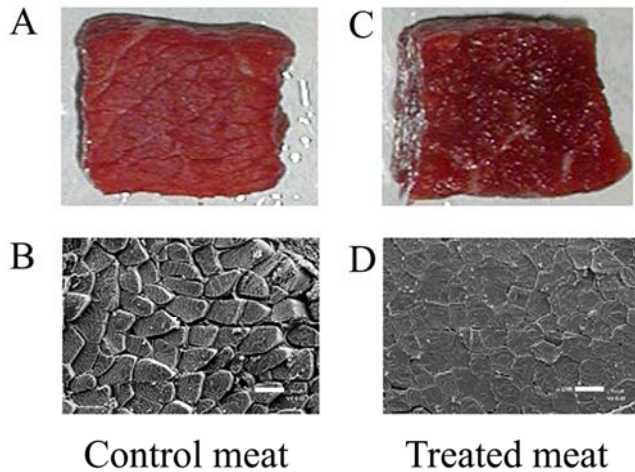


Figure 4. Appearance of frozen-thawed raw meats treated with or without salt-bicarbonate solution. Meats frozen after treatment with or without salt-bicarbonate solution and were thawed in a cold room at 4°C. (A) represents control meat; (B) represents SEM photograph of control meat; (C) represents salt-bicarbonate treated meat; and (D) represents SEM photograph of salt-bicarbonate treated meat. Each bar in (B, D) indicates 50 μm.

in salt-bicarbonate treated meat (Balcerzak et al., 2001). Salt-bicarbonate solution treatment seemed to damage slightly the structure of connective tissue during chilling and frozen storage, as evident by photographs shown in Figure 3E and 3F.

The salt-bicarbonate treated FT meat showed gummy characteristic, while the control FC meat appeared to be somewhat dry (Figure 4A and 4C). The reason for the adhesive surface is most likely to be attributed by the partial solubilization of myofibrillar proteins by the solution having high pH and salt concentration, of which the composition is similar to that of Weber and Edsall solution used for extraction of actomyosin. This fact was also confirmed by SEM observation (perpendicular to muscle fiber; Figure 4B and 4D) indicating that the surface of treated meat was smooth and sticky paste, whereas control meat showed the lamelliform surface having a distinct boundary with each muscle fiber.

When sample meats were cooked, many cracks ran in a reticular pattern on the surface of cooked control meat and it apparently shrank as a whole compared to the treated meat (Figure 5A and 5D). SEM photograph also showed a paste-like surface in the treated meat after cooking (Figure 5F). This trend, undoubtedly, reflects the gel of myofibrillar proteins (i.e. actomyosin gel) solubilized by the solution having high pH and salt concentration. Air pockets were observed in places of the cooked treated meat (Figure 5F) as have already pointed out by Sheard and Tail (2004). This was visible to the naked eye (Figure 5E). This air filled pocket may be of carbon dioxide, which is produced during the heating of bicarbonate in meat. The occurrence of

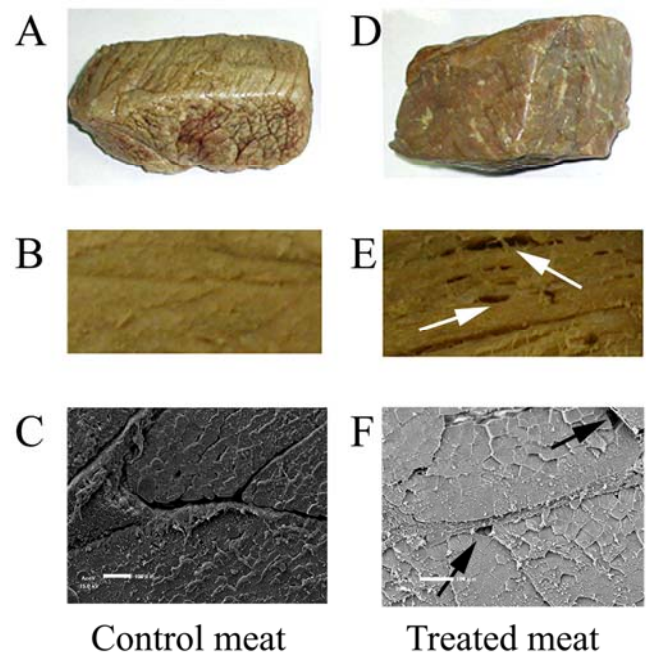


Figure 5. Appearance of cooked meats treated with or without salt-bicarbonate solution. Digital camera photographs of cooked meat (A, D); optical photographs of frozen meats treated with or without salt-bicarbonate solution were cooked after thawing (B, E) and low magnification SEM photographs of cooked meat (C, F). Here, (A) appearance of control meat; (B) optical picture of control meat; (C) SEM photograph of control meat; (D) appearance of salt-bicarbonate treated meat; (E) optical picture of salt-bicarbonate treated meat; and (F) SEM photograph of salt-bicarbonate treated meat. The arrows in (E, F) indicate the air-filled pockets around fiber bundles and longitudinal splits. Each bar in SEM photograph indicates 100 μm.

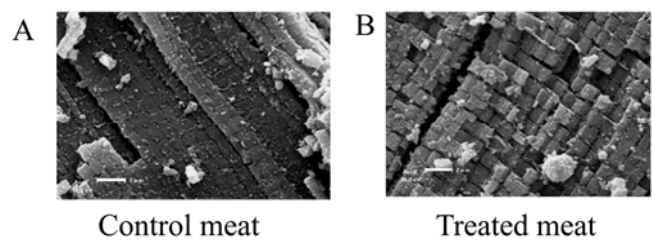


Figure 6. High magnification SEM photographs of frozen meat treated with or without salt-bicarbonate solution were cooked after thawing. (A) control meat; (B) treated meat. Each bar in SEM photograph indicates 2 μm.

carbon dioxide among the muscle bundles might also cause the disruption of muscle tissue. Interestingly, high magnification SEM photograph made it clear that sodium chloride-sodium bicarbonate treatment fractionated myofibrils vigorously and loosened among them (Figure 6). There is no obvious explanation for this observation, but it is possible that the high pH and salt concentration in salt-bicarbonate solution might have caused partial solubilization of myofibrillar proteins, leading to the

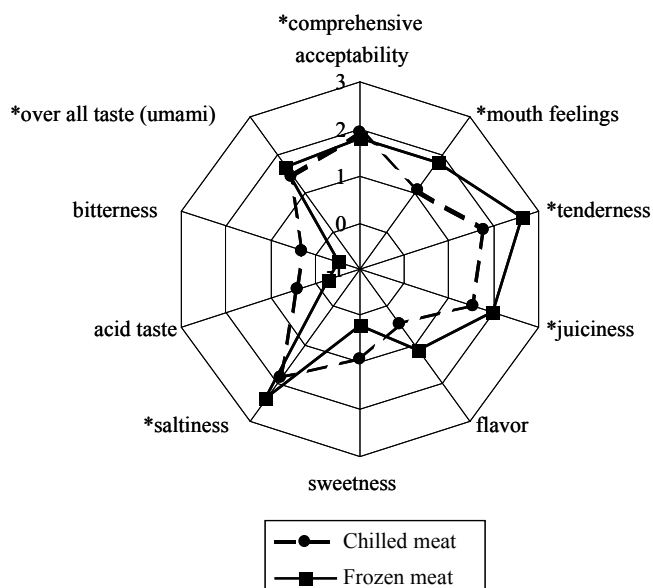


Figure 7. Radar chart of sensory evaluation test of control and treated meats after grilling. The details of the evaluation are described in the text. Star (*) indicates the significant difference ($p < 0.05$) between control and treated meats. ● represents chilled meat, ■ represents frozen meat.

weakness of myofibril structure. These morphological changes in connective tissue and myofibril may be considered to be the major cause of the reduction in the texture (hardness) and shear force in salt-bicarbonate treated meat.

Sensory evaluation

Ten items regarding the palatability of treated meats were evaluated by 24 panelists and the results were analyzed by one sample sign test (Figure 7). Panelists estimated that the treated meat was desirable on ten items irrespective of chilling or freezing. In particular, the rank of comprehensive acceptability in marinated meat was significantly higher than control. The mouth feelings, tenderness and juiciness were also higher in treated meat as expected. The presence of sodium chloride in the marinade contributed to high evaluation of saltiness and 'umami'. According to the report of Baublits et al. (2005), the addition of sodium chloride in beef *biceps femoris* gave the higher tenderness ratings compared to control. Higher juiciness in marinated meat might be due to the higher moisture content and water holding capacity in meat. The evaluation of tenderness coincided with the results of shear force (Figures 2 and 7). There was no significant difference in evaluations of sweetness, bitterness and acid taste between control and treated meats. Kuffman et al. (1998) reported that injection of sodium bicarbonate and salt into hot boned loins from gilts improved the flavor. An appearance of air pockets around the fiber bundles seemed to be little affecting the acceptability of treated meat as

judged with the result of sensory test.

The taste panel detected little difference in all evaluation items between chilling and freezing storage. Therefore, freezing after sodium chloride-sodium bicarbonate treatment is found to be useful for preparing "ready to cook" meat having high meat quality. Furthermore, the taste and flavor of meat can be altered by modifying the composition of this marinade solution depending on the purposes.

CONCLUSION

Based on the present study it can be concluded that the marinade solution consisting of sodium chloride and sodium bicarbonate help to decrease drip loss with increasing WHC in *biceps femoris* muscle. It also can reduce cook loss and shear force values with the ultrastructural changes which results in more softy and juicy taste during sensory evaluation in tough and hard beef *biceps femoris* muscle. All of these results will be informative and helpful for commercial users who are in problem of merchandising the tough cuts of beef muscle. Additionally, the restaurants, hospitals and fast food shops, which are in need of convenient high quality "ready to cook" meat, can apply these techniques. However, more detailed researches are needed, to establish new techniques for tenderization of tough cut muscle, combining sodium chloride-sodium bicarbonate and enzyme treatment under freezing, chilling or high pressurization conditions.

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