



Effect of Calcium Lactate on Physico-Chemical Characteristics of Shank Bone Extract

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Abstract

This study was conducted to develop calcium-fortified shank bone extract (SBE) and to determine the effect of adding calcium lactate on physico-chemical characteristics of SBE during cold storage. The following five experiment groups were used: Control (0%, no addition), T1 (0.05% calcium lactate), T2 (0.1% calcium lactate), T3 (0.5% calcium lactate), and T4 (1% calcium lactate). When the concentration of calcium lactate added to the SBE was increased, the pH, redness, and yellowness values were significantly reduced, whereas the salinity, sugar content, and turbidity of SBE were significantly increased. Sensory parameters such as aroma, flavor, and overall acceptability in the control, T1, and T2 had similar scores. The TBARS values of SBE was significantly increased when 1% of calcium lactate was added, and the VBN values of SBE with calcium lactate at day 7 were higher than that of control ($p < 0.05$). However, the addition of calcium lactate showed an inhibition effect on the growth of total microbial counts in SBE until 4 d of storage. The calcium content of SBE was increased by the addition of calcium lactate in a dose-dependently manner. The proper addition level of calcium lactate in the SBE was determined to be 0.1%.

Keywords calcium lactate, shank bone extract, sensory, total microbial counts

Introduction

Recently, beef consumption per capita in Korea was steadily increased from 7.5 kg in 2008 to 10.8 kg in 2014 (Ministry of Agriculture, 2015). To produce beef, cattle should be slaughtered, consequentially generate by-products from beef production. However, demand for by-products such as organs, head, leg, tail, bone, and blood by consumers has been decreased compared to the number of supplies and producers in Korea (Jeon, 2013). According to a report of Song *et al.* (2015), the prices of cattle leg and head fell 36% and 25.4%, respectively, in 2014 compared to those in 2012. In addition, eating habit in the modern society has been changed to well-being and ready to eat (RTE) foods due to increase numbers of single-person household and dual-income families. Long-time food such as soup made from by-products at home by oneself is not in large demand anymore (Moon *et al.*, 1998). Additionally, in terms of hygiene, quality, and production process,

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standards for by-products are not well established compared to meat. Furthermore, import of cheap meat products has accelerated the reduction in the consumption of by-products (Song *et al.*, 2015).

'Tang' or 'Gomtang' made from beef bones are one of famous foods as a traditional soup. It has been acknowledged as healthy food for pregnant women, lactation women, children, and weak people in the old times (Seol and Jang, 1990). These soups have been recommended for those people as a major source of calcium supply, and they contain a variety of minerals (calcium, potassium, sodium, phosphorus, ferrus and magnesium) (Kim *et al.*, 2014; Seol and Jang, 1990). Calcium is the most deficient nutrient in the diet of Koreans (Lee and Cho, 2004), but it is involved in a variety of body functions such as the contraction and relaxation of muscles, nerve transmission, blood coagulation, and other actions (Berchtold *et al.*, 2000; Douglas and Rubin, 1961; Furie and Furie, 1988). If human body lacks calcium, the growth will be delayed, which can increase the risk of rickets, osteomalacia, and osteoporosis (Choi and Pu, 2015). However, calcium actually exists in a low amount in the soups of beef bone. The bones are usually eaten as a type of broth, however compared to other food that does not eat in the form of broth, calcium contents in soups of tail, shank, and rib bones are 7-18 mg/100 g, which are much lower than those of shrimp (1,341 mg/100 g), anchovy (1290 mg/100 g), sea mustard (149 mg/100 g), or milk (91 mg/100 g) (NIAS, 2011). To make bone soups, bones are usually heated for a long time (more than 7-8 h) in Korea. A boiling time of 12 h is suitable for high extraction efficiency of nutritional contents and high sensory evaluation score (Kim, 2006). However, when nutrients from bones are increased by heating for a long time, phosphorus content will be extracted more than calcium content in bone soups (Kim, 2006; Seol and Jang, 1990). According to the report of Jang (2016), among the main foods consumed by Koreans, the Ca / P ratio was much higher in Kimchi, seasoned vegetables, vegetables, seaweed, and fruits (2.91-1.61) than meat, fish products, rice cake, salted foods, and alcohols (0.15-0.05). It has been reported that the intake of high amounts of phosphorus can reversely emit calcium from the body (Calvo, 1993), and Bell *et al.* (1977) reported that skeletal loss occurs when the intake of phosphorus increases and the value of Ca / P becomes less than 0.5.

Previously, bone extraction related researches have been performed the effects of the portion of bone, extraction

time, cattle breeds, and age on the characteristics of bone soups (Cho and Jung, 1999; Kim *et al.*, 2014; Seol and Jang, 1990). No study has determined the effect of calcium addition to bone extract characteristics, and calcium-fortified SBE have not yet been developed. Therefore, the objective of this study was to determine the effect of adding calcium lactate on the physico-chemical characteristics of SBE and find appropriate addition level of calcium lactate, as well as finally, to develop calcium-enriched SBE.

Materials and Methods

Preparation of shank bone extract (SBE)

Commercial Hanwoo shank bone was purchased from a local market. Visible impurities, subcutaneous debris, and excessive connective tissues were removed from the shank bone. The shank bone was washed three times with drinking water. After discarding the water, the extraction of shank bone was performed by modifying method of Choi *et al.* (2016). The extraction process was started by adding 2.5 L distilled water to 1 kg of shank bone followed by boiling over medium heat for 24 h using home electric range. After the extraction, the extract was adjusted to 2 liter distilled water and filtered using cotton stuff followed by cooling at 4°C for 6 h in a cold chamber. Fat on the supernatant was removed and calcium lactate (Junsei Chemical, Japan) was added into 300 mL of the liquid based on experimental treatments (Control; no addition, T1; 0.05% calcium lactate, T2; 0.1% calcium lactate, T3, 0.5% calcium lactate, and T4; 1% calcium lactate). The SBEs with calcium lactate were mixed well. The extraction process and experiment were repeated three times. The final gelatinized solutions were used for analysis of physicochemical (pH, salinity, turbidity, instrumental color, sensory evaluations, and mineral contents) and storage properties (TBARS, 2-thiobarbituric acid), VBN, volatile basic nitrogen, and TMC, total microbial count). The samples were placed in plastic container which can be closed with a cap and stored in a refrigerator (4°C) for 7 d, and used for analysis on the every test day (0, 4, and 7 d).

Methods of physicochemical analysis

pH

About 10 mL of SBE was added to 90 mL of distilled water, and the slurry was made using a homogenizer (T25B, IKA Sdn, Bhd., Malaysia) and the pH was measured using a pH meter. The pH meter was calibrated daily with stan-

andard buffers of pH 4.0 (9863 pH buffer solution, Mettler Toledo, Switzerland) and 7.0 (9865 pH buffer solution, Mettler Toledo, Switzerland) at 25°C.

Salinity and sugar content

The sample was filtered using Whatman No. 1 filter paper, and the salinity and sugar content of filtered samples were determined using a digital salimeter (PAL-03S, ATAGO, Japan) and saccharometer (PAL-3, ATAGO, Japan), respectively.

Viscosity

The viscosity values of samples were measured using a Viscometer (RVDV-II+P, Brookfield, USA) set at 10 rpm for 1 min. Samples were warmed at 40°C in a double boiler using a waterbath (BF-25B, Biofree, Korea). The spindle (No. 2) was positioned in a 100 mL metal cup filled with sample. The viscosity was measured in triplicates.

Turbidity

The turbidity was measured using a double beam spectrophotometer (Optizen 3220UV, Mecasys, Korea). The sample was filtered using filter paper (Whatman No. 1), and the turbidity of sample was measured at 590 nm. Distilled water was used as a blank. Turbidity is expressed as % transmittance.

Color

The CIE lightness (L^*), redness (a^*), and yellowness (b^*) values of sample were measured using a Minolta colorimeter (CR-400, Konica Minolta, Japan) using a 8 mm aperture size, illuminant D65, a 2° Closely matches CIE 1931 Standard Observer and measurement / illumination area $\Phi 8$ mm/ $\Phi 11$ mm. The instrument was standardized using a white plate ($L^* = 93.5$, $a^* = 0.3132$, $b^* = 0.3198$) before measurements. After one layer of plastic wrap covered on the measuring head of the colorimeter, the measuring head was placed in a specimen cup of 100 mL sample. Measurements were performed 5 times in the central part of the samples.

Sensory evaluation

Sensory evaluation was performed by 16 semi-trained panelists. The panel consisted of 10 researchers and 6 technicians at Chungbuk National University (male: female: 1:1; age range, 25-45 years old). Each sample was given as a random number. Samples were served randomly. Samples were warmed in a microwave oven (MR-400M,

Goldstar, Korea) for 1 min and served in paper cups per 50 mL at 40-45°C. The panel evaluated each treatment within each replication in triplicates. The color, aroma, flavor, and overall acceptability (1 = extremely undesirable, 5 = extremely desirable) of the extracts were evaluated using a five-point scale.

Storage characteristics

2-Thiobarbituric acid reactive substance (TBARS), volatile basic nitrogen (VBN), and total microbial count (TMC) of the shank bone extracts were investigated during storage at 4°C for 7 days. TBA test was used to determine the degree of lipid oxidation of the samples during storage at 4°C (Witte *et al.*, 1970). The samples (10 mL) were homogenized with 15 mL of cold 10% perchloric acid and 25 mL of distilled water using a stomach lab blender (model 400, Seward, UK) for 10 s at 1000 rpm, and the homogenate was filtered using filter paper (No. 1 Whatman International Ltd., UK). The filtrate (5 mL) was mixed, vortexed with 5 mL of 0.02 M thiobarbituric acid solution, and stored under dark-cold conditions for 16 h. The upper layer of each sample mixture was read using a spectrophotometer (Optizen-3220UV, Mecasys, Korea) at 529 nm and lipid oxidation was expressed as mg malondialdehyde per kg sample. To investigate protein deterioration, VBN was determined with some modifications (Pearson, 1976) using a Conway unit (Shibata Co. Ltd, Japan). VBN was expressed as mg per 100 g sample. Ten milliliters of sample was mixed with 90 mL of 0.1% peptone solution and homogenized using a stomach blender. The homogenate was serially diluted and spread on plate count agar (Difco Laboratories, USA). Plates were incubated at 37°C for 48 h (APHA, 1985) and then microbial counting was performed. The levels are reported as colony forming units (CFU) per ml of sample.

The contents of phosphorus and calcium

The contents of the phosphorus and calcium were determined following the method of AOAC (2000). Briefly, 5 mL of each sample was burned by dry ashing in a microwave ashing oven for 12 h with a final temperature of 600°C. The ash was dissolved in 10 mL of HCl and distilled water (1:1 v/v) solution followed by filtering through Whatman filter paper (No. 6) (AEC Scientific Co., Korea). The Ca (422.7 nm), and P (470 nm) were determined by atomic emission spectrophotometer ICP-OES (Spectro Analytical Instruments GmbH, Germany). A calibration curve was prepared for each element.

Statistical analysis

The entire experiment was replicated three times at different times in the same place. A completely randomized block design was used. The data of physico-chemical properties of extracts during storage were analyzed by an analysis of variance (one-way ANOVA) and two-way ANOVA was conducted to analyze the storage characteristics of extracts by the addition level and storage period using the General Linear Model procedure of SAS program. Significant differences among the samples were analyzed with Duncan's Multiple Range test at $p < 0.05$. Mean values and standard deviations of the means were reported. All data analyses were performed using SAS for Windows, version 9.1.3 (SAS, 2003).

Results and Discussion

Quality characteristics

The quality characteristics of SBE with calcium lactate added are presented in Table 1. The pH of SBE was significantly decreased ($p < 0.05$) with increased calcium lactate. The pH of control (6.82) without addition of any calcium lactate was the highest, but the pH of T4 with 1% calcium lactate added was the lowest among treatment groups ($p < 0.05$). The salinity of SBE was significantly increased ($p < 0.05$) as the amount of calcium lactate added was increased. Especially, the addition of more than 0.5% calcium lactate significantly increased ($p < 0.05$) the salinity compared to control. The sugar content and turbidity were also significantly increased ($p < 0.05$) in the SBEs with 0.5% and 1% calcium lactate added (T3 and T4, respectively). The viscosity of SBE was not affected by the addition of calcium lactate, even at concentration of 1%. The lightness values of SBE were significantly different ($p < 0.05$) among treatment groups. However, a consistent tendency

was not observed according to the amount of calcium lactate added. The redness and yellowness values of SBE were lower ($p < 0.05$) in T3 and T4 (0.5% and 1% of calcium lactate added, respectively) than the Control, T1, and T2 (0%, 0.05%, and 0.1% of calcium lactate added, respectively) ($p < 0.05$). It has been reported that bone contains a lot of minerals such as calcium, phosphorous, magnesium, and sodium (Park, 1986; Seol and Jang, 1990). When these minerals are dissolved in water, these exist in ionized state. The mineral contents in bone extract can also vary depending on the parts of the bone and the extraction time (Kim *et al.*, 2014; Park, 1986). In general, calcium lactate is used as additives in order to regulate acidity and fortify nutrient in foods. When 1 g of calcium lactate is dissolved in 20 mL of water, the pH of the solution ranges from 6 to 8 (MFDS, 2013). The crucial reason of decrease in pH of SBE might be due to the binding of lactic acid to calcium. However, this was not examined in details in this study. Pathomrungsriyounggul *et al.* (2010) have reported that the pH value of soy-milk fortified with 25 mM calcium lactate is significantly reduced compared to the control without any addition of calcium lactate. According to Lee *et al.* (2013), the addition of 2% liquid calcium to soy sauce seasoning can significantly decrease their redness and yellowness values compared to the control. In this study, the increase of calcium lactate added to SBE also increased the salinity, sugar content, and turbidity values. In general, salinity and sugar content are measured with refractometers. It is commonly used as a convenient instruments for measurement of Brix or salt concentration by weight of all soluble solids in solutions such as fruits, fruit juice, and similar products. However, these instruments are influenced not only by the target substance in a product but also by such substances as fruit acid and minerals (Dongare *et al.*, 2015; Meetan and North,

Table 1. Effect of calcium lactate on quality characteristics of shank bone extract

Treatments*	Control	T1	T2	T3	T4	
pH	6.82±0.02 ^a	6.80±0.01 ^b	6.75±0.01 ^c	6.19±0.01 ^d	6.08±0.01 ^e	
Salinity (%)	1.76±0.05 ^c	1.76±0.05 ^c	1.93±0.11 ^{bc}	2.10±0.17 ^b	2.36±0.05 ^a	
Sugar content (Brix, %)	2.33±0.20 ^b	2.30±0.01 ^b	2.33±0.05 ^b	2.73±0.23 ^a	2.96±0.05 ^a	
Turbidity (%)	1.97±0.01 ^b	1.94±0.01 ^c	1.91±0.01 ^d	2.26±0.01 ^a	2.26±0.01 ^a	
Viscosity (cP)	2.31±0.05	2.42±0.04	3.21±0.08	2.91±0.05	3.21±0.04	
Color**	CIE L*	34.59±0.54 ^a	32.57±0.45 ^b	32.06±1.12 ^b	33.78±1.46 ^{ab}	35.22±0.64 ^a
	CIE a*	-1.34±0.29 ^a	-1.14±0.10 ^a	-1.44±0.10 ^a	-1.86±0.19 ^b	-1.99±0.15 ^b
	CIE b*	3.09±0.42 ^b	3.82±0.34 ^a	3.21±0.16 ^b	1.91±0.42 ^c	1.52±0.17 ^c

*Control: 0%, no addition, T1: 0.05% calcium lactate, T2: 0.1% calcium lactate, T3: 0.5% calcium lactate, T4: 1% calcium lactate.

**L*: lightness, a*: redness, b*: yellowness.

^{a-c}Means±SD with different superscripts in the same row differ significantly ($p < 0.05$).

1991; 1995). Therefore, it is considered that T3 and T4 with high calcium lactate addition showed significantly high salinity and sugar content.

Sensory evaluation

The sensory evaluation results of SBE with added calcium lactate are shown in Table 2. The scores of subjective color in the control were significantly ($p<0.05$) higher than those of T2, T3, or T4. When the amount of calcium lactate added to SBE was increased, the color scores were decreased gradually. The aroma and flavor parameters were more acceptable ($p<0.05$) in the control, T1, or T2 compared to those in T3 or T4. Comprehensively, overall acceptability of SBE of T4 with 1% calcium lactate added was the lowest. The control and treatments T1 and T2 (with 0.05% and 0.1% calcium lactate added, respectively) had similar overall acceptability scores. In terms of sensory, the optimum addition level of calcium lactate was determined to be up to 0.1%.

Storage characteristics

The storage characteristics of SBE added with calcium lactate during 7 d of storage at 4°C are presented in Table

3. The TBARS values of SBE with 1% calcium lactate added (T4) were the highest during 7 d of storage. The values of VBN at day 0 was not different ($p>0.05$) among the treatments, however significant differences were observed ($p<0.05$) at days 4 and 7. On the day 4, the VBN values showed no consistent tendency according to the increase of calcium lactate, but the VBN values of treatments at day 7 was significantly ($p<0.05$) higher than the control. The addition of calcium lactate tended to decrease the TMC of SBE during cold storage. However, at the end of the storage periods (day 7), there was no significant ($p>0.05$) difference between treatment groups and the control. As the storage period increased, the storage characteristic values of all treatments including TBARS, VBN, and TMC increased significantly ($p<0.05$).

Lawrence *et al.* (2003) reported that calcium lactate acts as a pro-oxidant in the marinating beef loin with calcium lactate. Kim *et al.* (2012) have also reported that beef cut injected with 0.2 M calcium lactate solution has significantly higher TBARS values than control beef cut injected with water. In addition, anti-oxidant property of lactate has been reported (Choi and Chin, 2003; Groussard *et al.*, 2000). The anti-oxidative property of lactate is also reported when

Table 2. Effect of calcium lactate on sensory evaluation of shank bone extract

Treatments*	Control	T1	T2	T3	T4
Color	3.12±0.25 ^a	3.00±0.01 ^{ab}	2.62±0.25 ^{bc}	2.50±0.40 ^c	2.25±0.28 ^c
Aroma	3.12±0.25 ^a	3.00±0.01 ^a	3.00±0.01 ^a	2.87±0.25 ^{ab}	2.62±0.25 ^b
Flavor	3.12±0.25 ^a	3.12±0.25 ^a	3.12±0.25 ^a	2.75±0.50 ^{ab}	2.37±0.75 ^b
Overall acceptability	3.12±0.25 ^a	3.12±0.25 ^a	3.00±0.01 ^a	2.50±0.01 ^b	2.12±0.25 ^c

*Control: 0%, no addition, T1: 0.05% calcium lactate, T2: 0.1% calcium lactate, T3: 0.5% calcium lactate, T4: 1% calcium lactate. 1 = very unacceptable, 5 = very acceptable.

^{a-c}Means±SD with different superscripts in the same row differ significantly ($p<0.05$).

Table 3. Effect of calcium lactate on storage characteristics of shank bone extract

Treatments*	Control	T1	T2	T3	T4
TBA (mg malonaldehyde/1,000mL)					
0 d	0.26±0.13 ^{bc}	0.24±0.17 ^{bc}	0.36±0.12 ^{abB}	0.24±0.19 ^{bc}	0.44±0.09 ^{aB}
4 d	0.40±0.34 ^{bb}	0.44±0.14 ^{bb}	0.41±0.17 ^{bb}	0.46±0.14 ^{abB}	0.52±0.14 ^{aB}
7 d	0.84±0.31 ^{ca}	1.21±0.23 ^{ba}	1.02±0.21 ^{bcA}	0.87±0.05 ^{ca}	1.54±0.54 ^{aA}
VBN (mg%)					
0 d	6.45±0.27 ^C	6.36±0.31 ^C	5.81±0.41 ^B	6.08±0.15 ^C	6.17±0.72 ^B
4 d	7.68±0.34 ^{bb}	7.84±0.34 ^{ab}	6.95±0.65 ^{cb}	7.21±0.24 ^{bb}	7.62±0.82 ^{abB}
7 d	8.47±0.03 ^{ca}	10.54±0.36 ^{aA}	9.15±0.15 ^{ba}	9.84±0.15 ^{abA}	10.34±0.37 ^{aA}
Total microbial count (Log CFU/g)					
0 d	3.50±0.44 ^{ab}	1.73±0.05 ^{bc}	1.72±1.02 ^{bc}	2.40±0.08 ^{abB}	1.69±0.12 ^{bc}
4 d	4.61±0.05 ^{aA}	3.64±0.01 ^{bb}	3.84±0.54 ^{bb}	4.64±0.35 ^{aA}	3.21±0.35 ^{bb}
7 d	4.54±0.05 ^{abA}	4.64±0.65 ^{aA}	4.51±0.23 ^{abA}	4.97±0.34 ^{aA}	4.09±0.14 ^{ba}

*Control: 0%, no addition, T1: 0.05% calcium lactate, T2: 0.1% calcium lactate, T3: 0.5% calcium lactate, T4: 1% calcium lactate.

^{a-c}Means±SD with different superscripts in the same row differ significantly ($p<0.05$).

^{A-C}Means±SD with different superscripts in the same column differ significantly ($p<0.05$).

Table 4. Effect of calcium lactate on mineral contents of shank bone extract (mg/100g)

Treatments*	Control	T1	T2	T3	T4
P	154.69±0.25 ^a	131.92±2.91 ^b	121.59±1.02 ^d	126.36±3.65 ^c	97.12±0.30 ^e
Ca	3.70±0.03 ^e	67.49±0.07 ^d	116.45±1.10 ^c	527.66±8.64 ^b	1416.53±18.33 ^a

*Control: 0%, no addition, T1: 0.05% calcium lactate, T2: 0.1% calcium lactate, T3: 0.5% calcium lactate, T4: 1% calcium lactate.

^{a-e}Means±SD with different superscripts in the same row differ significantly ($p < 0.05$).

lactate is combined with phosphate. The anti-oxidative property of lactate is revealed in high pH after phosphate addition (Kim *et al.*, 2009). In this study, the reason for TBARS of T4 (1% calcium lactate) being the highest might be due to its lowest pH. It has been reported that calcium lactate also has inhibitory effect on the growth of microorganisms. Rahman *et al.* (2013) have reported that pork loin immersed in 3% calcium lactate solution has lower total viable count than the control (meat without immersing in calcium lactate solution). Chen and Shelef (1992) and Lawrence *et al.* (2003) have reported similar results. Shelef and Potluri (1995) have reported that lactate, rather than calcium ion, might be the principal antimicrobial factor because lactate can reduce the pH and chelation of essential components for bacterial growth.

The contents of phosphorus and calcium

The mineral contents of SBE with calcium lactate added are shown in Table 4. The control showed the highest ($p < 0.05$) phosphorus content, while T4 had the highest ($p < 0.05$) calcium content. As the amount of added calcium increased, the content of phosphorus decreased ($p < 0.05$) and the content of calcium increased ($p < 0.05$) significantly. Calcium and phosphorus are important factors for bone metabolism. Calvo (1993) has reported that the intake of foods with high phosphorus content but low calcium content can cause secondary hyperparathyroidism and bone loss. Kemi *et al.* (2010) have also reported that excessive intake of phosphorus compared to calcium can be deleterious to the bone. In addition, habitual diet with low Ca:P ratio may interfere with calcium metabolism and mineral metabolism. They recommended a Ca:P ratio higher than 0.5. In this study, Ca:P ratios in all treatments with calcium lactate exceeded 0.5. However, Ca:P ratio of the control was approximately 0.02.

Conclusion

As the addition of calcium lactate to SBE increased, microbial growth and the ratio of calcium and phosphorus were positive in terms of safety and health. However, it

had some negative effects on most of the physico-chemical properties of SBE. As results, considering the quality, storage characteristics, and Ca:P ratio of SBE, the addition level of calcium lactate was determined to be appropriately 0.1%.

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