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Different effect of sodium chloride replacement with calcium chloride on proteolytic enzyme activities and quality characteristics of spent hen chicken soup

ABSTRACT

Sodium chloride (NaCl) replacement with calcium chloride ($CaCl_2$) effect on protein solubility, proteolytic enzyme and quality characteristics of a chicken soup prepared from spent hen chicken were investigated. By means of immerse marination prior to cooking, a total of 60 skinless spent hen breast meat were randomly allocated into ten groups admitted to treatments with marinade solution containing Sodium tripolyphosphate (STPP) and reduced percentage of NaCl with $CaCl_2$ at 0, 25, 50, 75, and 100% at 4±2℃ for 20 hours. STPP was adjusted to 0.5% for all treatments and NaCl replacement at 0% was used as control. The different methods, particularly boiling at 100℃ and retorting at 121℃, 1.5 kgf/cm^2 for 60 minutes, were applied following marination. An upregulation of cathepsin-B and caspase-3 enzymes were a consequences from a higher percentage of $CaCl_2$ within meat environment. Accordingly, modified the protein solubility in particular the myofibrillar and total protein solubility. In addition, a significant increase in water holding capacity (WHC), pH value, myofibril fragmentation index (MFI), and moisture content was obtained due to salt replacement ($p<0.05$). Limited effect was observed for shear force value, collagen content and cooking yield. Eventually, this study implied that although protelytic enzyme and protein solubility was upregulated by the replacement of NaCl with $CaCl_2$ at >75%, extensive effect on texture properties was not observed. Therefore, NaCl replacement at 75% could be a promising strategy for quality improvement of spent hen chicken soup.

Keyword: Chicken soup, Spent hen chicken, $CaCl_2$, NaCl replacement, Proteolytic enzyme activities.

Introduction

Poultry meat consumption in South Korea has reached its steady-state and is expected to continuously grow for years. Among 986 million chicken slaughtered for consumption purposes in 2019, spent hen (SH) together with Korean native chicken accounted for only 6% of total production (USDA, 2020). Whereas, as reported by the Statistics Korea (KSIS, 2020), the number of egg-producing hen populations is approximately 70.89 million. Reckoning a number of reared SH chicken corresponds to a new productive pullets population. A similar number of SH chicken must be deducted simultaneously, leading to up to 25% of unutilized chicken. Consequently, SH chicken is taken as biological matter, with small portions utilized as soup and stew raw materials and feed purposes (Ajuyah et al, 1992). Considering its potential as an affordable protein and bioactive peptides source with a broad range of functions, such as antioxidant, antihypertensive, immunomodulatory, and anticancer (Korhonen, 2009; Udenigwe, 2014; Li-Chan, 2015), providing additional value for SH chicken is, therefore, an obvious economically beneficial for the poultry industry.

Among efforts for quality improvement of SH meat, texture properties are essential factor. The organoleptic quality and repurchasing decision are influenced by synergistic variables, including surface color, taste, and tenderness. Yet, after subjected to processing stages, the tenderness dominantly dictates the eating satisfaction (Barido et al., 2020). The first limiting factor that determines SH meat's poor quality is its tough texture (Kouguchi et al., 2012). A widely applied method for texture improvement is marination. It is an inexpensive, less-space requirement, and easy to control method with a significant effect, mainly owing to sodium ions (Lyon et al., 2005). Sodium chloride has been extensively used as a marinade salt because of its versatility; among them are solubilization of meat protein, growth inhibition of pathogenic bacteria, and enrichment of desirable taste (Ruusunen and Puolanne, 2005).

Coupled with phosphate, marination with these salt mixtures resulting in a significantly improved meat flavor, juiciness, tenderness and a lower cooking loss (Kin et al., 2009).

Increasing consumer awareness of daily salt consumption encourages producers to innovate healthier meat products with reduced sodium content (Broadway et al., 2011). Since excessive sodium chloride consumption corresponds to the occurrence of hypertension, obesity and even worse cardiovascular disease (Barros et al., 2019). Ideal consumption of sodium chloride should be below 2 g/day (Desmond, 2006). However, considering the significant effect of sodium salt for producing desirable texture, water holding capacity, and acceptable flavor, replacing another salt is not as easy as turning palm. It has to be replaced by salt with similar properties to maintain quality and sensory acceptance of meat products (Song et al., 2020). Calcium chloride is a salt other than potassium chloride (KCl) mentioned to have a healthy-supporting property as NaCl replacer. A more potent ion property than sodium ion, and a calcium-specific effect on upregulation of tenderness-related enzymes had proven that $CaCl_2$ contributed to a more tenderization effect of chicken meat than NaCl (Nurmahmudi and Sams, 1997). However, the different character of flavor given to meat products affects a lower sensory acceptance by the consumer (Inguglia et al., 2017).

Traditionally, chicken soup is a delicacy in some Asian countries such as China, Japan, and Korea. In Korea, ginseng chicken soup is widely consumed for its immunomodulatory property that comes from a synergistic effect of used ingredients (Dong et al., 2013). Korean chicken soup is prepared from a whole chicken carcass that its abdominal is filled with dried Chinese date, ginseng, garlic, and glutinous rice. It is believed that consuming Korean chicken soup during the summer season resulting in healthy improvements (Jeong et al., 2012). A study by Jeong et al. (2020) mentioned that Korean chicken soup prepared from SH chicken had the lowest quality attributes compared to that of white semi broiler, commercial broiler, and Korean native chicken, respectively, mainly due to the poor sensory acceptance and tough meat. Therefore, this study aimed to investigate the tenderization mechanism and marination effect with reduced soduim chloride solutions on chicken soup prepared from SH chicken in combination with different processing methods.

Materials and methods

Chicken soup preparation

Considering the adjustment of SH carcass to the size of the retort pouch, this study only utilized breast meat for chicken soup. It was prepared from SH chicken (Hyline white, 72 weeks old) from 60 birds 24 hours postmortem. The breast meat were obtained from commercial slaughterhouse with an average weight of 100±15 g. Left breast samples were randomly allocated into ten different groups consist of control and treatments with different mixtures of salt content, as well as different processing method, particularly boiling and retorting. While the right breast samples were randomly submitted into storage experiments investigated the different responses of proteolytic enzymes after treatment with different salt mixtures measured at day 0, 1, 3 and 5. Each of grouped samples intially subjected to marination with various percentage of salt mixtures that shown in Table 1. The STPP percentage was adjusted to maximum addition of 0.5% for all treatment groups. Salt mixture solutions were adjusted at 50 mL per 100 g sample. Marination was taken place at a temperature of 4±2°C through immersion for 20 hours followed by alleviation of marinade solution from the meat by letting samples to stand for 30 minutes, then wiped by towel tissue.

Broth preparation and Chicken soup manufacturing

Broth was previously extracted from chicken soup broth pack containing *Astragalus membranaceus* root, Mulberry branch, *Kalopanax septemlobus* branch, licorice, Siberian ginseng, and salt that boiled for 45 minutes. Afterward, the broth pack was removed and the solution was filtered using a stainless mesh filter (8 mm). Marinated SH meat samples were brought into different processing stages. In term of boiling, each treatment group consisting six breast meats (6 replications) were boiled together with 1.5L prepared broth at 100°C for

60 minutes. While for retorting, each retort pouch filled with six marinated breast meat from the same treatment group, stuffed with 1.5L of prepared broth. The retort process was conducted at a temperature of 121℃ with a pressure of 1.5 kgf/cm^2 for 60 minutes, followed by cooling process for 30 minutes. Cooked samples were subsequently filtered by using stainless mesh filter (600 µm) which subsequently be analyzed for its quality characteristics.

Proximate composition

Perfectly ground samples were measured for its moisture, crude protein, crude fat, and ash content according to a method by the Association of Official Analytical Chemists (AOAC, 2002). Each samples were prepared in triplicate.

Cooking loss

Cooking loss is a weight loss percentage after subjected to processing stages. It was obtained by calculating the weight loss (Initial weight–weight after process) divided by initial weight.

Water holding capacity

Ground samples in triplicate was measured for its water holding capacity according to a method by Kristensen and Purslow (2001). It is a centrifugal method where 5 g of the sample was placed in a centrifuge tube equipped with a wire mesh, heated in a water bath at 75℃ for 30 min, and centrifugated with (CS-6R Centrifuge; Beckman Instruments Inc., Hialeah, FL, USA) at 980 g for 10 min. WHC is the ratio between total moisture content and remaining water content.

pH value

Homogenated samples in triplicate were prepared from 5 g of perfectly ground sample added with 45 mL of distilled water assisted by homogenizer (PH91; SMT Co., Ltd., Tokyo, Japan). The pH value was determined by a previously calibrated pH meter probe (Seven Easy pH; Mettler-Toledo GmbH, Schwerzenbach, Switzerland).

Lipid oxidation

The determination of lipid oxidation rate were by using 2-thiobarbituric acid reactive substances (TBARS) according to a method by Sinhuber and Yu. (1977) with a slight modifiction. Analyses were conducted in triplicate and TBARS value was expressed as mg of malondialdehyde (MDA)/kg) of meat.

Shear force value

The measurement of shear force value was performed by using a TA-XT2*i* Plus (Stable Micro Systems, Surrey, UK) on a cooked samples. Cooked samples in rectangular size of 1.5 cm × 1.5 cm was placed under the V blade and cut with a constant speed (assay parameters were: pretest speed: 2.0 mm/s; test speed: 1.0 mm/s; posttest speed: 10 mm/s). Each sample was repeated three times.

Myofibrillar fragmentation index

The determination of myofibrillar fragmentation index was referring to a method by Culler et al. (1978) with slight modifications. Each of the marinated samples was prepared in triplicate. After subjected to isolation with pre-cooled isolating buffer (0.01 mM EDTA, 20 mM K_3PO_4, 100 mM KCl, and 1.0 mM $CaCl_2$; pH was adjusted to 7.0), the optical density (OD) was measured at 540 nm by using spectrophotometer. The MFI was obtained by multiplying OD with 200.

Collagen content

The collagent content of marinated samples were analyzed based on a method by Jayasena et al (2013). After subjected to hydrolysis according to a method by Palka and Daun. (1999), Collagen conten was calculated by multiplying the hydroxyproline content by a factor of 7.25 and was expressed as mg/g. The insoluble collagent was also expressed as mg/g. All the analyses were repeated three times.

Protein solubility

Protein solubility was determined according to procedures of Joo et al. (1999). Sarcoplasmic proteins were extracted from 2-g minced muscle using 20 ml of ice-cold 0.025 M potassium phosphate buffer (pH 7.2). While the total protein (sarcoplasmic + myofibrillar) was extracted from 2-g muscle using by 40 ml of ice-cold 1.1 M potassium iodide in 0.1 M phosphate buffer (pH 7.2). Myofibrillar protein solubility concentrations were obtained by calculate the difference between total and sarcoplasmic protein solubility.

Proteolytic enzyme activities

To understand the possible responses of tenderness-related enzymes (calpains, cathepsin-b and caspase-3 enzyme) to treatment with different percentage of sodium chloride replacement, the activity of endogenous enzymes were measured on sixty raw SH breast meat after subjected to marination with various percentage of sodium chloride : calcium chloride at a temperature of 4±2°C through immersion for 20 hours, samples were then allocated to storage and determine its change on enzyme activities at day 0, 1, 3 and 5, wherein day 0 was used as reference data. according to a method described by Broadway et al. (2011) with slight modifications, in which a substrate (Suc-LY-AMC) was used for calpains, while ARR-AFC was used for determination of cathepsin-B enzyme activity. To obtain the supernatants, 0.5 g of the homogenated samples in 1.0 mL lysate (100 mmol/L HEPES, 20% glycerol, 0.5 mmol/L EDTA, 5.0 mmol/L DTT, and 0.2% SDS; pH57.5) were centrifuged at the speed of 12,000 rpm for 20 min. Regarding the control, the supernatant was replaced with ddH_2O. The absorbance value of the mixture was detected at 380 nm/460 nm (excitation/emission) for calpains and 400 nm/505 nm (excitation/emission) for cathepsin-B. The enzyme activity is shown as the relative absorbance value per min, per mg to the control. In term of Caspase-3 activity was determined according to a method described by He et al. (2019). A total of 1.0 mM Ac-DEVD-pNA (dissolved in DMSO) was used as the substrate and incubated with the supernatant at 37 ℃ for 1 h. The absorbance value of the mixture was detected at a wavelength of 405 nm, and caspase-3 enzyme activity is expressed as the relative absorbance value per min, per mg to the control.

Statistical analysis

The data analyses in this study performing R-version 3.6.1 (The R-foundation for Statistical Computing, Vienna, Austria), CRAN mirror and library USA (CA 1) equipped with Agricolae. two-way multivariate analysis of variance (MANOVA) was used to determine the effect of sodium chloride replacement as well as different manufacturing method on investigated variables. Significant value of the mean for each group was continuesly analyzed using the Duncan's multiple range test, with a consideration as significant for *p*-value lower than 0.05.

Results and Discussions

Proximate composition

The effect of sodium chloride replacement with calcium chloride on chicken soup breast meat made from spent hen chicken is shown in Table 2. Moisture percentage was significantly affected by treatments, with the highest percentage of NaCl replacement, resulting in a significantly higher moisture percentage of the breast meat ($p<0.05$). However, the replacement of NaCl with $CaCl_2$ until 75% did not contribute to an increased moisture content ($p>0.05$). Furthermore, crude protein, crude fat and ash content were not significantly affected by different salt mixture percentage in marinade solution ($p>0.05$). Besides, regardless of the concentration of NaCl replacement, meat processed in a high temperature and high-pressure condition (retort) significantly lower moisture content ($p<0.05$) with a less effect on crude protein, crude fat and ash content compared to that processed by boiling ($p>0.05$). Increased in moisture percentage might be due to the high ionic strength of calcium ion that promotes a more protein-ion bond and consequently assembles a layer-like form on the meat surface to act as a water repulsion inhibitor (Nurmahmudi and Sams, 1997). Coupled with an osmotic-like force with a high affinity to interact and pull water molecules into the meat environment is another hypothesis from the effect of chloride ion on imparting free protein with negative charges (Lee et al., 2012). Following these studies, a replacement of salt type with a stronger ionic strength may lead to an increased of moisture percentage.

Cooking loss, WHC and pH value

Different processing methods significantly affected the shrinkage percentage of spent hen chicken soup in all treatment samples. As shown in Table 3, breast meat processed by retorting promoted a significantly higher cooking loss compared to that processed by boiling

(p<0.001). No significant effect was observed after marination with reduced sodium chloride, indicating a similar calcium chloride attribute to prevent cooking loss as sodium chloride did. In term of WHC, breast meat samples marinated with a solution containing STPP with 100% $CaCl_2$ resulting in a higher WHC percentage compared to that containing 100% NaCl (p<0.05), meaning a higher portion of $CaCl_2$ within marinade solution significantly contributed to an increased ability of the muscle to retain water, a factor for the increment of WHC (Barido et al., 2020b). Different cooking methods did not significantly affect WHC, wherein spent hen meat samples processed through boiling did not differ from that processed by retorting (p>0.05).

A similar trend was also observed for pH value, the value increment was found to be significantly affected by a higher percentage of $CaCl_2$ within marinade solution (p<0.001). Replacement of NaCl with ≥ 50% $CaCl_2$ resulting in a spent hen breast meat chicken soup with a significantly higher pH value (p<0.001). In addition, along with a significantly lower pH value of chicken soup breast meat processed by retorting compared to that of boiled chicken soup, this study found a significant interaction between treatment and processing method on pH value (p<0.001).

Numerous studies considered WHC to be an important variable that strongly correlated with meat tenderness. It characterizes the moisture retention ability by meat during processing as well as water uptake (Barido et al., 2020a). A higher affinity and ionic strength of $CaCl_2$ compared to NaCl promotes a greater protein extractability and consequently drive to a more protein-ion bond with negative charges. Although $CaCl_2$ and NaCl may share similar ion, the tenderization mechanism may differ, wherein calcium chloride could provide faster tenderization via similar conductivity level of ionic strength as sodium chloride, as well as calcium ion-specific effect to activate more proteolytic activities (Koohmaraie et al., 1989). The more assembly of protein-ion filament on meat surface is also consequence by calcium chloride, this condition is therefore assumed to increase the inner meat environment ability to retain water and significantly contribute to an increased WHC. (Nurmahmudi and Sams, 1997). Furthermore, this result also confirmed a previous study by Bowker and Zhang. (2015), that categorized breast meat with a higher WHC promoted to a higher pH value, and vice versa.

TBARS value

Table 3 displays the concentration of malondialdehyde content, a highly reactive compound as a marker for lipid oxidation. Different NaCl:$CaCl_2$ composition within the marinade solution did not significantly contribute to different lipid oxidation rates (p>0.05). However, regardless the percentage of NaCl replacement, samgyteang breast meat processed by retorting significantly resulting in a higher lipid oxidation rate than that of boiled chicken soup (p<0.05). This finding was in accordance with a previous study by Kim et al. (2020), wherein a higher manufacturing condition (temperature and humidity) has a strong interaction with a faster increment of lipid oxidation. TBARS value in this study, however, was still below the threshold in which meat product is characterized as off-flavor and unacceptable by consumer (Nam and Ahn, 2003). These results implied that even complete replacement of NaCl with $CaCl_2$ salt within the marinade solution did not bring a detrimental effect on meat quality, particularly the formation of lipid oxidation.

Shear force value and MFI

The changes in the tenderness level of chicken soup breast meat after treatment with different NaCl:$CaCl_2$ are shown in Table 4. Although an inconsistent effect on shear force value was observed, the lower shear value tended to result from a higher portion of $CaCl_2$ within the marinade solution. In addition, higher sodium chloride replacement with calcium chloride promoted a significantly higher fragmentation of myofibrillar protein ($p<0.001$). In the other hand, the MFI value was significantly affected by both manufacturing method and salt replacement. A higher replacement percentage of NaCl with $CaCl_2$ significantly increased the MFI value ($p<0.001$), while accompanied by a higher value in chicken soup breast meat processed through retorting compare to that of by boiling ($p<0.001$). A decrease in meat tenderness results from a complex interaction between myofibrillar protein thick filament called myosin and actin. Once an animal is sacrificed, biochemical reactions within muscle promote an increased crosslinking between actin and myosin to form actomyosin. The higher formation of actomyosin would generate meat that is characterized as stiff (Broadway et al., 2011). In addition, MFI is another widely used index to determine the occurrence of postmortem tenderization in meat (Volpelli et al., 2005). The degradation of myofibrillar protein is strongly associated with architectural changes in meat muscle, wherein a higher MFI value indicates a higher proteolytic activity. The MFI value in this study significantly affected by $CaCl_2$ percentage within the marinade solution might be due to the calcium ion effect. The calcium ion was proven to regulate the calpains activity (Nurmahmudi and Sams, 1997), thus a higher concentration of calcium ion leading to a more calpains activity that consequently causes fragmentation of myofibrillar protein and destruction of muscle integrity. This result confirmed a previous study by Li et al. (2017) that mentioned a high concentration of $CaCl_2$ salt significantly contributed to a higher MFI value of goose breast meat.

Collagen content

Table 4 displays the effect of marination with different percentage of NaCl:CaCl on chicken soup breast meat made from spent hen chicken. Both total collagen and insoluble collagen content were not differ among samples ($p>0.05$). Different processing methods also did not significantly modify the collagen content from spent hen breast meat ($p>0.05$). Kong et al. (2008) mentioned that along with the muscle integrity, total and insoluble collagen content dominantly dictate the tenderness level of meat. At least two factors influence thermal residual collagen and unextractable collagen; advancing age and different chicken breeds (Nakamura et al., 1975). A study by Jeong et al. (2020) characterize among various chicken breeds used as chicken soup raw material, spent hen meat was denoted to exert a higher collagen content and, consequently, affect a lower sensory acceptance. While in this study, marination with a diverse percentage of NaCl and $CaCl_2$ prior to cooking did not generate a spent hen breast meat with significantly lower collagen content. It was proposed that the calcium-specific effect regulates the intra-cellular calpain activity with substrate limited to tropomodulin protein (Li et al., 2017). Therefore, protein with a large molecular size such as collagen was assumed to not be affected by these events.

Protein solubility

Total protein solubility was significantly affected by NaCl:$CaCl_2$ percentage within marinade solution. A replacement of NaCl with $CaCl_2$ at ≥50% resulting in a significantly higher total protein solubility among treatments as seen in Table 5, with the highest was observed for complete replacement of NaCl with $CaCl_2$ (p<0.001). Similar trend was also found for myofibrillar protein. A significant effect was found for myofibrillar protein solubility of spent hen breast meat after marinated with 50:50% (NaCl:$CaCl_2$), as well as 100% marination with $CaCl_2$ (p<0.001). Retorting process seemed to impart a significant increase of both total soluble protein and myofibrillar protein solubility of chicken soup breast meat compare to that of boiling groups (p<0.001). The soluble sarcoplasmic protein concentration was not affected by either marination with NaCl replacement or a different processing method (p>0.05). Calcium-specific ion was assumed to regulate calpains' activity, wherein tropomodulin protein is a potential substrate that consequently promotes protein degradation

(Li et al., 2017). An increase in total soluble protein, as well as myofibrillar protein solubility observed by this study, confirmed a previous study by Nurmahmudi and Sams (1997) that besides its calcium specific effect, the ionic strength of $CaCl_2$ could promote a higher protein extractability and faster tenderization effect compared to NaCl on spent hen meat.

Proteolytic enzyme activities

The activity of cathepsin-B enzyme on spent hen breast meat after treated with salt mixtures containing different percentage of NaCl and $CaCl_2$ was shown in Fig 1. The cathepsin-B enzymes treated with 100% $CaCl_2$ was observed to had the highest activity among treatments at day 1 ($p<0.05$). Enzyme activities were shown to be not statistically different in treatment containing 75% and 100% $CaCl_2$ replacement. In addition, different salt mixtures effect was not differ among treatments at day 3 and day 5 ($p>0.05$). The upreglation of cathepsin-B enzymes activity treated with complete replacement of NaCl with $CaCl_2$ were recorded at 8-33%. It possibly due to the specific effect of Ca^{2+} ion from $CaCl_2$ that modified the activity of cathepsin-B. As mentioned by Li et al. (2017), $CaCl_2$ containing Ca^{2+} ion play a significant role for the tropomodulin protein activation (actin filament capping protein). The higher concentration of Ca^{2+} ion within meat environment resulting in a higher depolymerization rate of actin filament through the hydrolization of tropomodulin. These results confirmed previous study by He et al. (2019) who found a significant upregulation of cathepsin-B enzyme by $CaCl_2$ on duck breast meat.

Similar trends were observed on caspase-3 enzymes, enzyme responsible for the activation of apoptosis. The highest activity of caspase-3 enzymes found by this study was recorded on spent hen breast meat treated with NaCl replacement with $CaCl_2$ at >75% ($p<0.05$) at day 1, with a slight effect at day 3 and 5 respectively. Although the caspase-3 enzyme contributions during post-mortem tenderization are still debated and not clearly explained, studies by Nurmahmudi and Sams. (1997) revealed that this enzyme could be another important enzymes after calpains and cathepsin families for texture improvement in chicken meat. As well as study by He et al. (2019) who found that $CaCl_2$ could led to an upregulation of caspase-3 enzyme and eventually promoted to postmortem tenderization.

In term of calpains enzyme, the effect of different percentage of NaCl and $CaCl_2$ was determined not to differ among treatments throughout storage days, indicated that calpains enzyme activities could independently worked without interference of neither NaCl nor $CaCl_2$. This results on spent hen breast meat was consistent with that explained by He et al. (2019), who studied the effect of different enzyme responses after treated with $CaCl_2$ and MDL-28,170 on duck breast meat, and found no significant effect on the calpains enzyme activities. Moreover, the declining activies of the calpains enzyme throughout storage day was possibly a result of the restricted substrate that could be utilized for the enzyme activation.

Conclusion

Immerse marination of spent hen breast meat into five different solutions containing a diverse percentage of NaCl and $CaCl_2$ were performed by this study to evaluate the effect of salt replacement on quality characteristics of chicken soup prepared from spent hen chicken. Marination with a combination of STPP and $CaCl_2$ did not have detrimental effects on quality characteristics of spent hen breast meat chicken soup and maintaining a good quality as STPP and NaCl mixture did. Instead, a complete replacement led to a significantly increased WHC, pH value and MFI. An upregulation of cathepsin-B and caspase-3 enzymes were a consequences from a higher percentage of $CaCl_2$ within meat environment. Accordingly, modified the protein solubility in particular the myofibrillar and total protein solubility.

However, the salt replacement had a limited effect on texture improvement. Therefore, combined with retorting, marination with a marinade solution containing STPP and NaCl replacement with $CaCl_2$ at 75% is a promising strategy for the development of healthier chicken soup made from spent hen meat with improved quality.

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Table 1. Marinade mixture solution containing sodium tripolyphosphate, NaCl and $CaCl_2$

Treatments[1)]	STPP[2)] (%)	NaCl (%)	$CaCl_2$ (%)	NaCl replacement concentrations (%)
Control	0.5	0.5	0	0
Treatment 1	0.5	0.375	0.125	25
Treatment 2	0.5	0.25	0.25	50
Treatment 3	0.5	0.125	0.375	75
Treatment 4	0.5	0	0.5	100

[1)]NaCl replacement percentage with $CaCl_2$, Control (breast meat marinated with 100% NaCl), Treatment 1(breast meat marinated with 75% NaCl:25% $CaCl_2$), Treatment 2 (breast meat marinated with 50% NaCl:50% $CaCl_2$), Treatment 3 (breast meat marinated with 25% NaCl:75% $CaCl_2$), Treatment 4 (breast meat marinated with 100% $CaCl_2$).

[2)] STPP, Sodium tripolyphosphate

Table 2. Proximate composition of samgyetang breast meat marinated with reduced NaCl with $CaCl_2$ prior to cooking in different manufacturing method

Variable	Cooking method	NaCl replacement percentage[2)]					SEM[1)]	Sample[3)]	Method[4)]	Sample x Method[5)]
		0%	25%	50%	75%	100%				
Moisture (%)	boiling	67.14^{bx}	67.87^{bx}	68.25^{bx}	68.99^{bx}	70.73^{ax}	0.39	<0.05	<0.05	0.75
	retorting	66.86^{bx}	66.60^{by}	66.98^{by}	67.73^{aby}	69.47^{ay}	0.32			
Protein (%)	boiling	29.78	29.12	28.50	28.95	28.32	0.76	0.74	0.88	0.49
	retorting	29.27	30.06	28.74	28.40	28.55	1.37			
Fat (%)	boiling	2.25	2.03	2.34	2.04	2.19	0.17	0.97	0.69	0.51
	retorting	2.31	2.23	2.46	2.07	2.18	0.09			
Ash (%)	boiling	0.78	0.79	0.79	0.79	0.77	0.01	0.85	0.90	0.82
	retorting	0.80	0.81	0.82	0.78	0.80	0.02			

[a-b]Mean values within the same row indicating a significant different following NaCl replacement with $CaCl_2$.

[x-y]Mean values within the same column indicating a significant different following different manufacturing method.

[1)]SEM, standard error of the mean.

[2)]NaCl replacement percentage with $CaCl_2$, 0%(breast meat marinated with 100% NaCl), 25%;(breast meat marinated with 75% NaCl:25% $CaCl_2$), 50%(breast meat marinated with 50% NaCl:50% $CaCl_2$), 75%(breast meat marinated with 25% NaCl:75% $CaCl_2$), 100%(breast meat marinated with 100% $CaCl_2$).

[3)]Sample, significance value after treatment with different NaCl replacement percentage.

[4)]Method, significance value after treatment with different manufacturing method.

[5)]Sample x Method, the interaction between different NaCl replacement percentage with different manufacturing method

Table 3. Meat quality of samgyetang breast meat marinated with reduced NaCl with $CaCl_2$ prior to cooking in different manufacturing method

Variable	Cooking method	NaCl replacement percentage[2)]					SEM[1)]	Sample[3)]	Method[4)]	Sample X Method[5)]
		0%	25%	50%	75%	100%				
WHC (%)	boiling	67.21^{b}	66.98^{b}	68.11^{ab}	72.12^{ab}	74.33a	1.05	<0.01	0.97	0.99
	retorting	67.08^{b}	67^{b}	68.34^{ab}	71.98^{ab}	74.15a	1.36			
pH	boiling	5.98^{bx}	5.98^{bx}	6.08^{ax}	6.01^{bx}	6.07^{ax}	0.01	<0.001	<0.001	<0.001
	retorting	5.66^{cy}	5.64^{cy}	5.75^{by}	5.80^{ay}	5.79^{ay}	0.02			
Cooking loss (%)	boiling	18.25^{by}	19.17^{aby}	19.76^{ay}	18.14^{by}	18.12^{by}	2.34	0.11	<0.001	0.17
	retorting	28.64^{bx}	29.75^{abx}	30.11^{ax}	29.23^{bx}	29.04^{bx}	1.88			
TBARS (mg MDA/kg)	boiling	0.92^{y}	0.89^{y}	0.87^{y}	0.97^{y}	0.95^{y}	0.06	0.85	<0.05	0.031
	retorting	1.27^{x}	1.15^{x}	1.24^{x}	1.29^{x}	1.14^{x}	0.04			

$^{a\text{-}b}$Mean values within the same row indicating a significant different following NaCl replacement with $CaCl_2$.

$^{x\text{-}y}$Mean values within the same column indicating a significant different following different manufacturing method.

1)SEM, standard error of the mean.

2)NaCl replacement percentage with $CaCl_2$, 0%(breast meat marinated with 100% NaCl), 25%;(breast meat marinated with 75% NaCl:25% $CaCl_2$), 50%(breast meat marinated with 50% NaCl:50% $CaCl_2$), 75%(breast meat marinated with 25% NaCl:75% $CaCl_2$), 100%(breast meat marinated with 100% $CaCl_2$).

3)Sample, significance value after treatment with different NaCl replacement percentage.

4)Method, significance value after treatment with different manufacturing method.

5)Sample x Method, the interaction between different NaCl replacement percentage with different manufacturing method

Table 4. Tenderness-related traits of samgyetang breast meat marinated with reduced NaCl with $CaCl_2$ prior to cooking in different manufacturing method

Variable	Cooking method	NaCl replacement percentage[2)]					SEM[1)]	Sample[3)]	Method[4)]	Sample X Method[5)]
		0%	25%	50%	75%	100%				
Shear force value (kgf)	boiling	2.30^{ab}	2.46^{a}	2.35^{ab}	2.24^{ab}	2.19^{b}	0.03	<0.001	0.16	0.97
	retorting	2.28^{ab}	2.39^{a}	2.27^{ab}	2.20^{b}	2.16^{b}	0.02			
MFI	boiling	29.33^{cy}	30.33^{bcy}	32.33^{by}	35.00^{ay}	36.66^{ay}	0.59	<0.001	<0.001	0.5
	retorting	36.00^{cx}	37.34^{bcx}	41.66^{abx}	43.67^{ax}	41.67^{abx}	0.90			
Total collagen (mg/g)	boiling	1.72	1.58	1.67	1.64	1.78	0.02	0.49	0.93	0.33
	retorting	1.68	1.63	1.75	1.69	1.62	0.03			
Insoluble collagen (mg/g)	boiling	0.79	0.76	0.77	0.77	0.79	0.00	0.1	0.45	0.11
	retorting	0.80	0.77	0.79	0.77	0.77	0.00			
Total soluble protein (mg/ml)	boiling	91.19^{cy}	91.97^{cy}	93.20^{cy}	97.02^{by}	101.63^{ay}	0.89	<0.001	<0.001	0.71
	retorting	103.86^{cx}	104.79^{cx}	106.33^{cx}	111.71^{bx}	116.33^{ax}	1.08			
Myofibrillar protein solubility (mg/ml)	boiling	17.08^{y}	17.12^{y}	17.06^{y}	17.10^{y}	17.53^{y}	0.32	0.69	<0.01	0.94
	retorting	18.55^{x}	17.88^{x}	17.98^{x}	18.68^{x}	19.35^{x}	0.30			
Sarcoplasmic protein solubility (mg/ml)	boiling	74.11^{cy}	74.85^{cy}	76.14^{cy}	79.91^{by}	84.10^{ay}	0.81	<0.001	<0.001	0.85
	retorting	85.31^{bx}	86.91^{bx}	88.35^{bx}	93.04^{ax}	96.98^{ax}	0.99			

[a-c]Mean values within the same row indicating a significant different following NaCl replacement with $CaCl_2$.

[x-y]Mean values within the same column indicating a significant different following different manufacturing method.

[1)]SEM, standard error of the mean.

[2)]NaCl replacement percentage with $CaCl_2$, 0%(breast meat marinated with 100% NaCl), 25%;(breast meat marinated with 75% NaCl:25% $CaCl_2$), 50%(breast meat marinated with 50% NaCl:50% $CaCl_2$), 75%(breast meat marinated with 25% NaCl:75% $CaCl_2$), 100%(breast meat marinated with 100% $CaCl_2$).

[3)]Sample, significance value after treatment with different NaCl replacement percentage.

[4)]Method, significance value after treatment with different manufacturing method.

[5)]Sample x Method, the interaction between different NaCl replacement percentage with different manufacturing method

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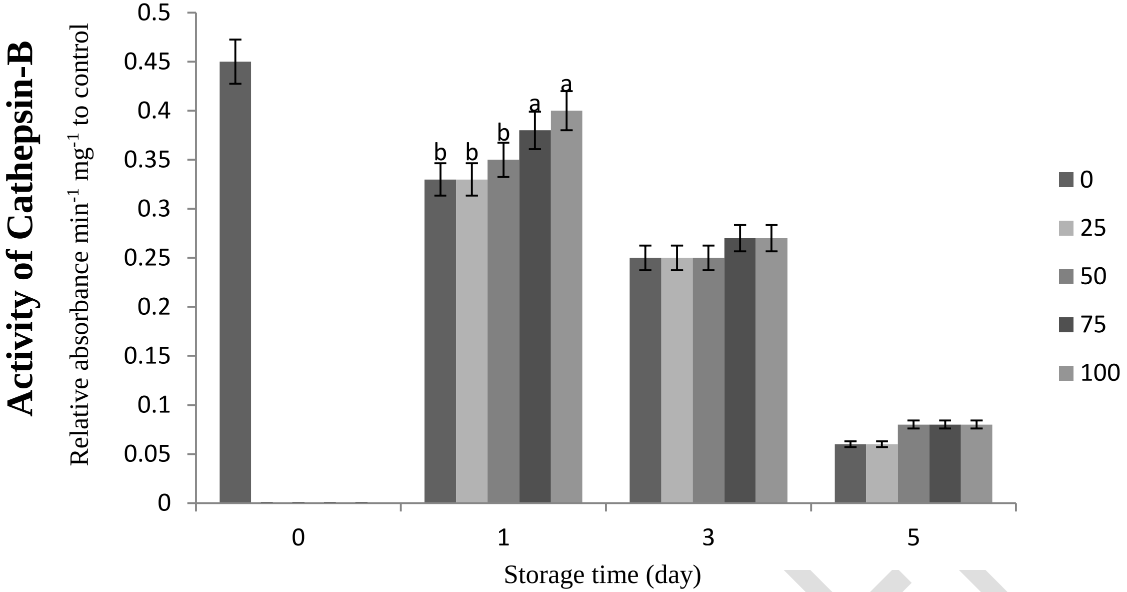
Activity of Cathepsin-B
Relative absorbance min-1 mg-1 to control
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
b
b
b
a
a
0
1
3
5
Storage time (day)
0
25
50
75
100

Fig 1. Cathepsin-B enzyme activities expressed in (relative absorbance value per min, per mg to control) of spent hen breast meat after treated with different salt solution containing different percentage of NaCl:$CaCl_2$, 0%(breast meat marinated with 100% NaCl), 25%; (breast meat marinated with 75% NaCl:25% $CaCl_2$), 50%(breast meat marinated with 50% NaCl:50% $CaCl_2$), 75%(breast meat marinated with 25% NaCl:75% $CaCl_2$), 100%(breast meat marinated with 100% $CaCl_2$).

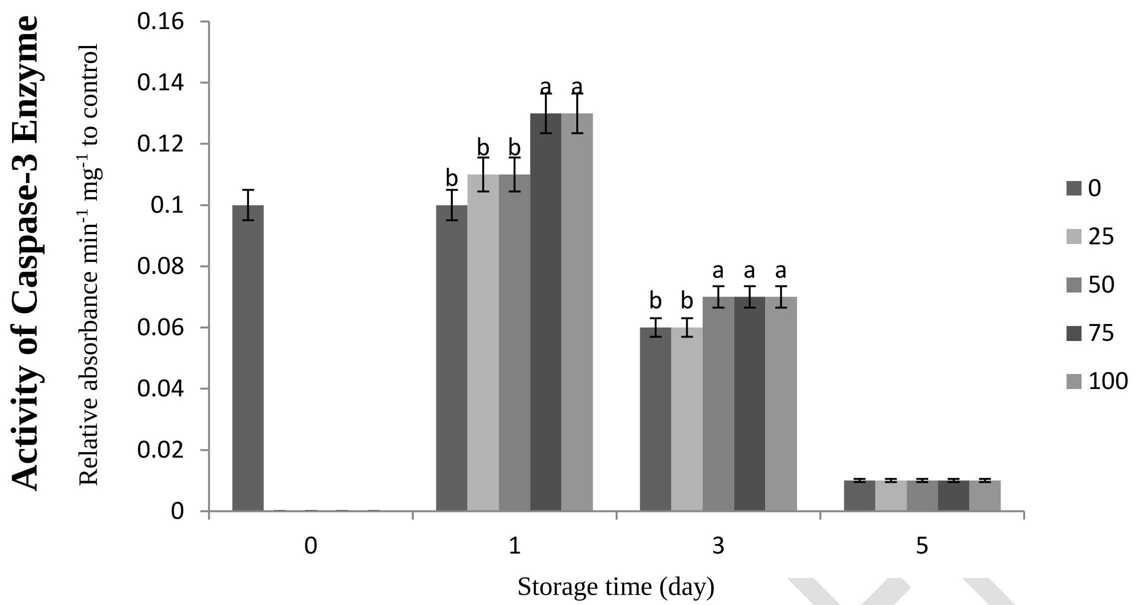

Fig 2. Caspase-3 enzyme activities expressed in (relative absorbance value per min, per mg to control) of spent hen breast meat after treated with different salt solution containing different percentage of NaCl:$CaCl_2$, 0%(breast meat marinated with 100% NaCl), 25%; (breast meat marinated with 75% NaCl:25% $CaCl_2$), 50%(breast meat marinated with 50% NaCl:50% $CaCl_2$), 75%(breast meat marinated with 25% NaCl:75% $CaCl_2$), 100%(breast meat marinated with 100% $CaCl_2$).

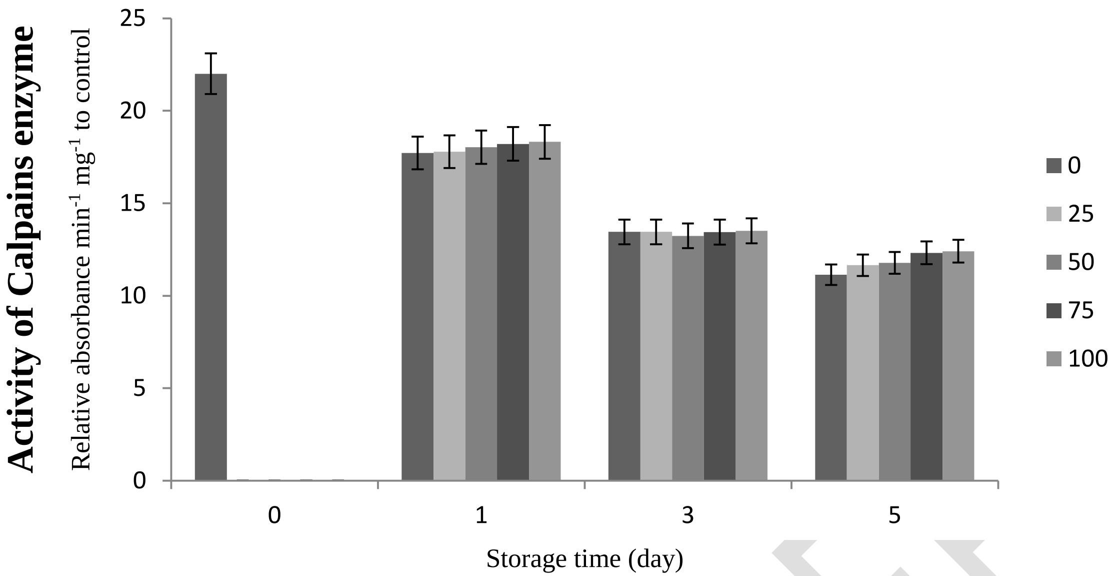

Fig 3. Calpain enzyme activities expressed in (relative absorbance value per min, per mg to control) of spent hen breast meat after treated with different salt solution containing different percentage of NaCl:$CaCl_2$, 0%(breast meat marinated with 100% NaCl), 25%; (breast meat marinated with 75% NaCl:25% $CaCl_2$), 50%(breast meat marinated with 50% NaCl:50% $CaCl_2$), 75%(breast meat marinated with 25% NaCl:75% $CaCl_2$), 100%(breast meat marinated with 100% $CaCl_2$).