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Effect of Pre-rigor Salting Levels on Physicochemical and Textural Properties of Chicken Breast Muscles

Hyun-Wook Kim, Ko-Eun Hwang, Dong-Heon Song, Yong-Jae Kim, Youn-Kyung Ham,
Eui-Joo Yeo, Tae-Jun Jeong, Yun-Sang Choi[1], and Cheon-Jei Kim*

Department of Food science and Biotechnology of Animal Resources, Konkuk University, Seoul 05029, Korea
[1]*Food Processing Research center, Korean Food Research Institute, Seongnam 13539, Korea*

Abstract

This study was conducted to evaluate the effect of pre-rigor salting level (0-4% NaCl concentration) on physicochemical and textural properties of pre-rigor chicken breast muscles. The pre-rigor chicken breast muscles were de-boned 10 min post-mortem and salted within 25 min post-mortem. An increase in pre-rigor salting level led to the formation of high ultimate pH of chicken breast muscles at post-mortem 24 h. The addition of minimum of 2% NaCl significantly improved water holding capacity, cooking loss, protein solubility, and hardness when compared to the non-salting chicken breast muscle ($p < 0.05$). On the other hand, the increase in pre-rigor salting level caused the inhibition of myofibrillar protein degradation and the acceleration of lipid oxidation. However, the difference in NaCl concentration between 3% and 4% had no great differences in the results of physicochemical and textural properties due to pre-rigor salting effects ($p > 0.05$). Therefore, our study certified the pre-rigor salting effect of chicken breast muscle salted with 2% NaCl when compared to post-rigor muscle salted with equal NaCl concentration, and suggests that the 2% NaCl concentration is minimally required to ensure the definite pre-rigor salting effect on chicken breast muscle.

Keywords: chicken breast, post-mortem, pre-rigor salting, physicochemical properties, rigor-mortis

Introduction

Pre-rigor muscle, which signifies the biochemical muscle state before rigor-mortis, is generally obtained from hot boning or accelerated processing techniques. The pre-rigor muscle has been known to have several economic benefits, including reduction in labor and storage facility and superior processing quality due to the higher level of adenosine tri-phosphate (ATP) content and pH value when compared to post-rigor muscle (Pisula and Tyburcy, 1996). With the passage of time after slaughter, unfortunately, the pre-rigor muscle starts to lose functional properties due to depletion of ATP and a decrease in pH with the accumulation of lactic acid by anaerobic glycolysis (Hamm, 1977; Pisula and Tyburcy, 1996).

For these reasons, previous studies have been mainly focused on the maintenance and improvement of the functional properties of the pre-rigor muscle. Especially, it is well known that the addition of salt into the pre-rigor muscle, which is called as "pre-rigor salting", improved the extraction of myofibrillar proteins, such as myosin heavy chain and actin (Abu-Bakar *et al*., 1989; Bernthal *et al*., 1989; Bernthal *et al*., 1991). Coon *et al*. (1983) suggested that the pre-rigor salting caused the high ultimate pH values, similar to living body, due to rapid passage of rigor-mortis, as well as the reported principle effect of salt. The improved water holding capacity and protein solubility resulted from the extended space by disassociation of myofibrillar proteins under the high pH value (Hamm, 1977). Thus, numerous studies have examined the pre-rigor salting effect on the processing quality and determining the optimal salt level, which can lead to the pre-rigor salting effect, in mammalian species, such as mainly beef and pork (Hamm, 1981; Puolanne and Terrell, 1983).

Recently, modern consumers interested in their health have preferred to consume white muscle, which has lower fat and higher protein contents compared to red muscle (Jones, 1992). The consumption of chicken meat,

*Corresponding author: Cheon Jei Kim, Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 05029, Korea. Tel: +82-2-450-3684, Fax: +82-2-444-6695, E-mail: kimcj@konkuk.ac.kr

which is a typical white muscle, is constantly increasing all over the world. According to a report of Korea Poultry Association (2014), per capita consumption of chicken meat in Korea has risen steadily from 2003 (7.9 kg) to 2012 (11.5 kg). Nevertheless, consumption patterns of chicken meat were too simple and were mainly limited to mostly whole meat, meat cuts, nuggets, and patties. Choi *et al*. (2009) indicated that the reason for the limitation was related to the inadequate processing quality of chicken compared to those of beef and pork. To enhance the processing quality of chicken meat, thus, the application of pre-rigor salting technique to chicken muscle carried an important meaning. Previously, Karakaya *et al*. (2005) reported the excellent cooking yield of pre-rigor chicken muscle and suggested its applicability to pre-rigor salted chicken. Choi *et al*. (2009) reported that pre-rigor salted chicken breast had a higher water holding capacity and a lower cooking loss than the post-rigor salted chicken breast. However, there is a lack of research on the determination of minimal salt concentration, which can ensure the pre-rigor salting effect on chicken muscle, and the investigation on the processing characteristics of chicken muscles by the difference in added salt levels.

Therefore, the objective of this study was to (1) evaluate the effect of pre-rigor salting levels in terms of the sodium chloride (0-4% NaCl) and (2) determine the effect of pre-/post-rigor salting with 2% NaCl on physicochemical and textural properties of chicken breast muscles.

Materials and Methods

Preparation of raw materials

A total of 60 broilers (Arbor Acre Broiler, 5 wk of age and approximately 1.6-1.8 kg live weight) were obtained from a local poultry processor and transported to the meat science laboratory of Konkuk University, Seoul, Korea. The birds were slaughtered in accordance with the poultry slaughter procedure described by Alvarado and Sams (2000). Feed and water were allowed until 12 h and 2 h prior to slaughter, respectively. The birds were stunned electrically at 50 V for 10 s and killed by bleeding from a single unilateral neck for approximately 3 min. After bleeding and evisceration, chicken breast muscles (*Musculus pectoralis major*) from 50 broilers were obtained within 10 min after slaughter. And then, the obtained muscles were ground through an 8 mm plate within 20 min post-mortem and divide into five portions. For post-rigor treatments, chicken breast muscles were obtained

from remained 10 broilers 24 h after slaughter. Sodium chloride (NaCl) of 0, 1, 2, 3, and 4% (w/w, based on sample weight) was added to each portion, and then, the pre-rigor salted chicken breast muscles were mixed with blender for 3 min. Also, the post-rigor chicken breast muscle was salted with 2% NaCl after grinding (8 mm). Total time required was under 25 min until pre-rigor salting processing. The salted chicken breast muscles were individually vacuum-packaged into nylon/polyethylene bags and refrigerated at 4ºC for 24 h until analysis.

pH measurements

The pH values of sample were determined with a pH meter (Model 340, Mettler-Toledo GmbH, Switzerland) at post-mortem 25 min, 1, 2, 6, 12, and 24 h. The pH values of samples were measured by blending a 5 g sample with 20 mL distilled water for 60 s in a homogenizer at 8,000 rpm (Ultra-Turrax SK15, Janke & Kunkel, Germany).

Water holding capacity (WHC)

WHC was determined in triplicate by filter paper pressed method (Grau and Hamm, 1953). Sample of 0.3 g was weighed onto a Whatman No. 2 filter paper and pressed between two plexiglass plate for 3 min. The areas of pressed water and sample were measured using planimeter (Type KP-21, Koizumi, Japan). WHC was calculated as follows;

WHC (%) = area of pressed sample / area of pressed water × 100

Cooking loss

All samples stuffed into each centrifugal tube (approximately 50 g) and were cooked in a constant-temperature water bath (75°C, 30 min). The cooked samples were cooled to room temperature for 6 h. After cooling, the cooked samples were reweighed. Cooking loss was determined by calculating the weight differences before and after cooking as follows.

Cooking loss (%) = [(weight of raw sample (g) − weight of cooked sample (g)) / weight of raw sample (g)] × 100

Protein solubility

The solubility of the salt soluble (myofibrillar) protein was determined following the modification of procedures described by Saffle and Galbreath (1964). A 5 g sample

was blended with 50 mL 3% sodium chloride solution at 14,000 rpm for 2 min using homogenizer (AM-7, Nihonseiki Kaisha, Japan). The mixture was centrifuged at 3,000 g for 15 min. The protein concentration of supernatant was determined using the biuret method (Gornall et al., 1949) and using bovine serum albumin (Sigma Chemical Co., USA).

Myofibrillar fragmentation index (MFI)

Myofibrils was obtained according to the method of Olson et al. (1976) using MFI buffer (20 mM K_2HPO_4/KH_2PO_4, pH 7.0, 100 mM KCl, 1 mM EDTA, 1 mM NaN_3). The myofibrils were suspended in MFI buffer. An aliquot of myofibril suspension was diluted with the MFI buffer to 0.5 mg/mL protein concentration and the absorbance of this suspension measured at 540 nm. MFI values were recorded as absorbance units per 0.5 mg/mL myofibril protein concentration multiplied by 200.

Texture (hardness)

TPA was performed at room temperature with a texture analyzer (TA-XT2i, Stable Micro Systems, England). Cooked meat samples (2.5 cm in height, 2.0 cm in diameter) were taken from the central portion of each meat. Prior to analysis, samples were allowed to equilibrate to room temperature (20ºC, 3 h). The conditions of texture analysis were as follows: pre-test speed 2.0 mm/s, post-test speed 5.0 mm/s, maximum load 2 kg, head speed 2.0 mm/s, distance 8.0 mm, force 5 g. The calculation of hardness was obtained by graphing a curve using force and time plots.

2-Thiobarbituric acid (TBA) value

Lipid oxidation was assessed in triplicate by TBA method of Tarladgis et al. (1960) with minor modifications. A 10 g sample was blended with 50 mL distilled water for 2 min and then transferred to a distillation tube. The cup used for blending was washed with an additional 47.5 mL of distilled water, which was added to the same distillation flask with 2.5 mL 4 N HCl and a few drops of an antifoam agent (KMK-73, Shin-Etsu Silicone Co., Ltd., Korea). The mixture was distilled and 50 mL distillate was collected. 5 mL of 0.02 M 2-Thiobarbituric acid in 90% acetic acid (TBA reagent) was added to test tube containing 5 mL of the distillate and mixed well. The tubes were capped and heated in a boiling water bath for 30 min to develop the chromogen and cooled to room temperature. The absorbance was measured at 538 nm, against a blank prepared with 5 mL distilled water and 5 mL TBA-reagent, using a UV/VIS spectrophotometer (Optizen 2120 UV plus, Mecasys Co., Ltd., Korea). The TBA values were calculated as mg MDA/kg meat.

TBA (MDA mg/kg) = (optical density of sample − optical density of blank) × 7.8

Statistical analysis

All tests were done at least three times for each experimental condition and mean values were reported. One-way ANOVA was employed to determine the significance of main effect (pre-rigor salting level), and significance of difference between means of post-rigor 2% and pre-rigor 2% treatments (t-test) was determined using the SAS statistical package (2008). Duncan's multiple range test ($p<0.05$) was used to determine differences between treatment means. The procedure CORR of the SAS package was used to calculate correlations between the pre-rigor salting level and the measurements.

Results and Discussion

Changes in pH value

Generally, the decline in pH of meat after slaughter occurs due to the accumulation of lactic acid formed from the glycogen under anaerobic glycolysis (Hamm, 1977). The change in the pH value of pre-rigor chicken breast muscle salted with various NaCl levels after post-mortem twenty-four hours is shown in Fig. 1. The initial pH value of chicken breast at fifteen minutes after slaughter was 6.48, and then, the pH values of all treatments rapidly decreased until post-mortem two hours. The pH value of post-rigor 2% treatment was 5.85 (data not shown). According to Jones (1992), poultry muscle quickly reached the rigor-mortis compared to the mammalian species and began at about post-mortem one hour. Similar to our study, Yu et al. (2009) reported that hot-boned chicken breast muscle reached the ultimate pH within post-mortem three hours. After pre-rigor salting, the ultimate pH value of pre-rigor chicken breast muscle was gradually affected by the added amount of NaCl. The pre-rigor chicken breast muscle salted with 4% NaCl showed the highest ultimate pH value (6.05), but the pH value of non-salting chicken breast muscle was 5.78. Bernthal et al. (1989) reported that the changes in pH values of hot-boned ground beef was linearly related to the added amount of NaCl. Coon et al. (1983) indicated that the addition of NaCl on pre-rigor muscle could cause the high ultimate pH due to the speeding passage of rigor-
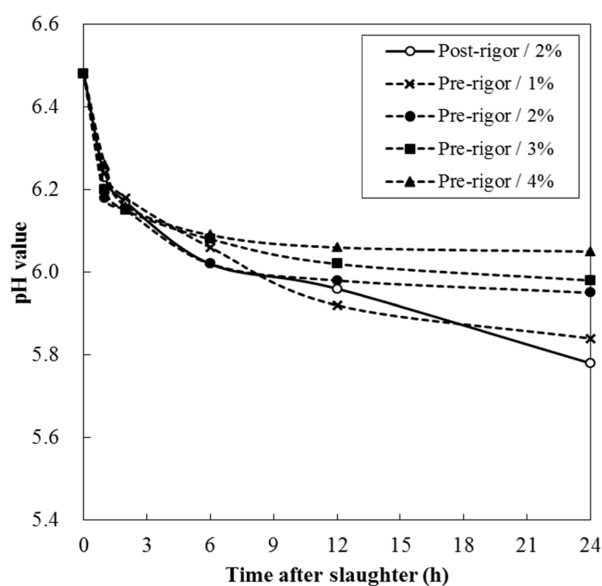
Fig. 1. Effect of pre-rigor salting levels on the changes in pH values of chicken breast muscles during post-mortem 24 h.
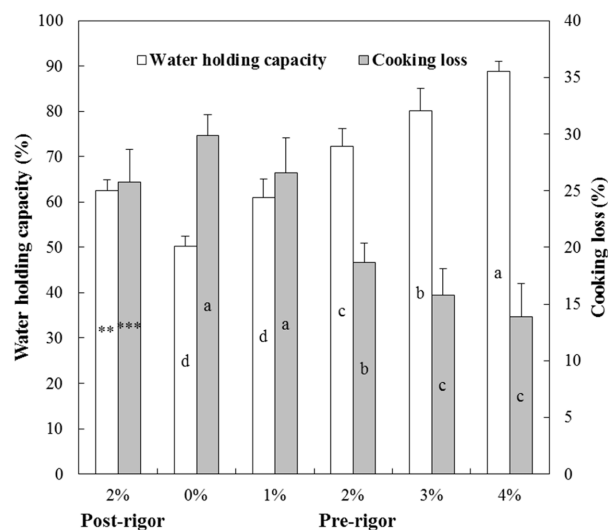

Fig. 2. Water holding capacity and cooking loss of pre-rigor chicken breast muscles salted with various sodium chloride (NaCl) levels at post-mortem 24 h. [a-d]Means in the samples with different letters (among pre-rigor treatments) are significantly different ($p<0.05$). Asterisk in bars means the significance of t-test between post-rigor 2% and pre-rigor 2%. **$p<0.01$; ***$p<0.001$.

mortis. Dalrymple and Hamm (1974) suggested that the inactivation of enzymes associated with glycolysis under high ionic strength and low pH, such as phosphorylase and phosphofructokinase, was one of the main reasons for the high ultimate pH of the pre-rigor salted muscle. Thus, an increase in ultimate pH of pre-rigor salted chicken breast muscle might be associated with the inactivation of glycolytic enzymes due to an increase in ionic strength.

Water holding capacity (WHC) and cooking loss

WHC of meat, which implies the ability to retain moisture within the internal muscle structure, in meat processing, is directly related to the release of moisture during manufacturing and thermal processing. In this respect, the pre-rigor salting has been recognized as one of the effective methods to maintain the WHC and cooking yield of pre-rigor muscle (Pisula and Tyburcy, 1996). The effect of pre-rigor salting level on WHC of chicken breast muscles at post-mortem twenty-four hours is shown in Fig. 2. WHC of pre-rigor chicken breast muscles increased with increasing added amounts of NaCl whereas the cooking loss decreased. However, the pre-rigor chicken breast muscle salted with 1% NaCl exhibited similar WHC and cooking loss when compared to non-salting chicken breast muscle ($p>0.05$). In addition, pre-rigor 2% NaCl treatment had a significantly higher water holding capacity ($p<0.01$) and lower cooking loss ($p<0.001$) than post-

rigor 2% treatment. Bernthal et al. (1989) reported that the highest level of salting concentration (4% NaCl) resulted in an excellent pre-rigor salting effect on ground beef and suggested that, minimally, 2% NaCl was needed to expect the pre-rigor salting effect. Pisula and Tyburcy (1996) reported that the excellent WHC of pre-rigor salted muscles results from its high pH value and ATP content. According to Boles and Swan (1997), the increase in salting level could influence the net charge of muscle proteins, which contributed to the changes in the solubility of muscle proteins. In this study, the addition of NaCl at a minimum of 2% began to function on the improvement of WHC of pre-rigor chicken breast muscles, resulting in the reduction in cooking loss.

Protein solubility, myofibrillar fragmentation index (MFI), and texture

The effect of pre-rigor salting level on protein solubility, MFI, and texture of chicken breast muscles at post-mortem twenty-four hours is shown in Table 1. As expected, the highest protein solubility was observed for the pre-rigor chicken breast muscle salted with 4%. However, there was no significant difference in the protein solubility between chicken breast muscles salted with 3% and 4% NaCl ($p>0.05$). The pre-rigor 1% treatment showed similar protein solubility to post-rigor 2% treat-

Table 1. Effect of pre-rigor salting levels on protein solubility, myofibrillar fragmentation index (MFI), and hardness of chicken breast muscles

Treatments (rigor state/NaCl)	Protein solubility (mg/g)	Myofibrillar fragmentation index (MFI)	Hardness (kg)
Pre-rigor/0%	8.24±2.40[1)]d	101.44±3.38[a]	4.51±0.40[c]
Pre-rigor/1%	12.24±1.08[c]	91.44±3.24[b]	5.97±0.38[b]
Pre-rigor/2%	14.65±2.26[b]	88.08±1.71[c]	7.14±0.44[a]
Pre-rigor/3%	15.66±1.53[a]	82.54±4.02[d]	7.43±0.38[a]
Pre-rigor/4%	16.06±2.12[a]	80.46±1.48[d]	7.45±0.25[a]
Post-rigor/2%	12.50±1.32	105.24±4.12	5.51±0.27
Significance of t-test[2)] (Post/2% *vs* Pre/2%)	***	**	**

[1)]All values are mean±standard deviation.
[2)]Significance of t-test between post-rigor/2% and pre-rigor/2%. **$p<0.01$; ***$p<0.001$.
[a-d]Means within a column (among pre-rigor treatments) with different letters are significantly different ($p<0.05$).

ment ($p>0.05$). Bernthal *et al*. (1989) reported that the addition of NaCl above 2% on pre-rigor ground beef could maintain the superior protein solubility, similar to the pre-rigor state, after rigor-mortis. In this study, only the addition of 1% NaCl showed an increase, in the protein solubility, against non-salted chicken breast muscle. Lan *et al*. (1995) reported that the myofibrillar protein content of chicken breast (12.57%) was lower than that of beef (12.65%). Moreover, McIntosh (1967) noted that the chicken muscle showed the low protein extractability during all times of post-mortem when compared to beef and pork. For these reasons, thus, the difference in minimum NaCl concentration, which can ensure the pre-rigor salting effect, might be associated with the myofibrillar protein content and the protein extractability of chicken breast muscle.

Generally, the myofibrillar protein degradation by endogenous proteases occurred on Z-disc in muscle after slaughter, and the degree of the degradation could be measured by MFI method (Olson and Parrish, 1977). In addition, MFI value was an important factor determining meat tenderness, along with sarcomere length, ionic strength, and animal species (Koohmaraie, 1994). The pre-rigor chicken breast muscle salted with 2% NaCl showed lower MFI value than post-rigor chicken breast muscle salted with 2% NaCl. Among pre-rigor treatments, the non-salting chicken breast muscle had a higher MFI value than all pre-rigor salted chicken breast muscles ($p<0.05$), and an increase in added amounts of NaCl led to a decreased MFI value. Sárraga *et al*. (1989) reported that the addition of NaCl suppressed the activity of endogenous proteases, such as calcium activated proteases and cathepsin. Thus, the decreasing MFI value with increasing added amount of NaCl could result from the inactivation of endogenous proteases due to an

increase in ionic strength.

As mentioned above, the results of protein solubility and MFI values, which is responsible for the textural properties of meat and meat product, have shown diverse results. In terms of the hardness, an increase in pre-rigor salting level increased hardness of pre-rigor chicken breast muscles after thermal processing, and pre-rigor 2% treatment had a significantly higher hardness than post-rigor 2% treatment. The hardness of the pre-rigor chicken breast muscle salted with 4% NaCl (7.45 kg) was about 1.65 times compared to that of non-salting chicken breast muscle (4.51 kg). Thus, this result suggested that an increase in hardness due to pre-rigor salting could be related to both protein solubility and MFI value.

2-thiobarbituric acid (TBA) value

Lipid oxidation, along with microbiological safety, is one of the major factors affecting the quality characteristics of meat and meat product during storage period. Chicken meat, which has high polyunsaturated fatty acids content compared to other animal species, is very sensitive to lipid oxidation (Rhee *et al*., 1996). In this study, an increase in the added amount of NaCl obviously caused the lipid oxidation of chicken breast muscles (Fig. 3). A minimum addition of 2% NaCl showed significantly increased TBA value compared to non-salting treatment ($p<0.05$); however, there was no significant difference in TBA values between 3% and 4% treatments (0.39-0.40 mg MDA/kg meat) ($p>0.05$). In addition, pre-rigor 2% treatment showed a significantly higher TBA value than post-rigor 2% treatment ($p<0.05$). According to Torres *et al*. (1988), the addition of more than 0.5% could promote the lipid oxidation of pre-rigor ground beef during storage. Lee *et al*. (1997) indicated that the addition of NaCl released the iron ion from the myoglobin, consequently
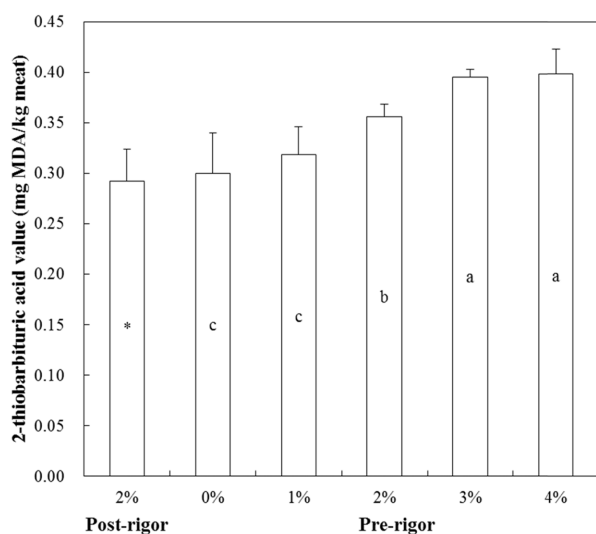
Fig. 3. 2-thiobarbituric acid (TBA) values of pre-rigor chicken breast muscles salted with various sodium chloride (NaCl) levels at post-mortem 24 h. [a-c]Means in the samples with different letters (among pre-rigor treatments) are significantly different (*p*<0.05). Asterisk in bars means the significance of t-test between post-rigor 2% and pre-rigor 2%. **p*<0.05.
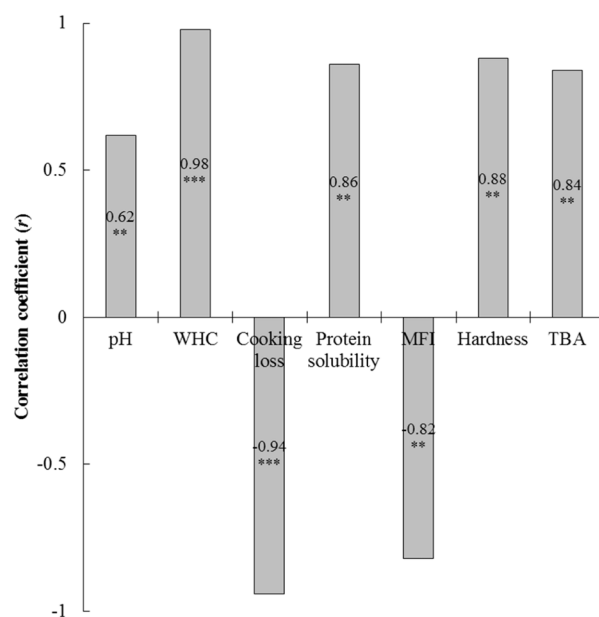

Fig. 4. Coefficients of correlation between pre-rigor salting levels (added amount of NaCl) and measurements of chicken breast muscles at post-mortem 24 h. WHC, water holding capacity; MFI, myofibrillar fragmentation index; TBA, 2-thiobarbituric acid value. The number and asterisk in a bar graph refer to Pearson correlation efficient and significant (**p*<0.01, ***p*<0.001), respectively.

accelerating the lipid oxidation. Rhee *et al*. (1996) suggested that frozen raw beef and pork meats showed higher TBA value than chicken meat because beef and pork meats contained high heme iron content. Thus, the reason why the lipid oxidation of pre-rigor salted chicken muscle was accelerated under high NaCl concentration, in comparison with beef muscle, could be greatly associated with the content of heme pigments. In the case of the production of meat product, Rhee *et al*. (1988) reported that the restructured nugget prepared with pre-rigor salted pork showed the acceleration of lipid oxidation when compared to that made with post-rigor salted pork. Even if the pre-rigor salting caused the acceleration of pre-rigor chicken breast muscles in our study, considering the consumption threshold (1.0 mg MDA/kg meat) related to lipid rancidity (Suh, 1984), it would be desirable that the pre-rigor salted chicken breast muscle was used as quickly as possible.

Correlation between pre-rigor salting level and measurements

The coefficients of correlation for pre-rigor chicken breast muscle salted with various NaCl concentrations are presented in Fig. 4. The relationships between pre-rigor salting level and ultimate pH value (*p*<0.01, r=0.62), WHC (*p*<0.001, r=0.98), protein solubility (*p*<0.01, r=0.86),

hardness (*p*<0.01, r=0.88), and TBA value (*p*<0.01, r=0.84) were positively significant whereas the relationships between pre-rigor salting level and cooking loss (*p*<0.001, r=-0.94) and MFI (*p*<0.01, r=-0.82) were negatively significant. Thus, this result showed that the NaCl concentration (0-4%) had a slightly linear relationship with the physicochemical and textural properties of pre-rigor chicken breast muscles.

Conclusion

The increase in pre-rigor salting level contributed to the formation of high ultimate pH of chicken breast muscles at post-mortem twenty-four hours. In addition, the significant pre-rigor salting effect, which could improve and/or maintain the WHC, cooking loss, protein solubility, and hardness, was observed for the chicken breast muscle salted with at least 2% NaCl, and this study certified the pre-rigor salting effect when compared to post-rigor muscle at equal salting concentration (2% NaCl). On the other hand, the increase in pre-rigor salting level caused the inhibition of myofibrillar protein degradation and the acceleration of lipid oxidation. However, the difference in NaCl concentration between 3% and 4% had no great

influence on either the positive or negative effects. Therefore, our results suggested that 2-3% NaCl concentration was properly required to ensure the pre-rigor salting effect on chicken breast muscle.

Acknowledgements

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