

## Effect of Ginger Extract and Citric Acid on the Tenderness of Duck Breast Muscles

Fu-Yi He, Hyun-Wook Kim, Ko-Eun Hwang, Dong-Heon Song, Yong-Jae Kim, Youn-Kyung Ham, Si-Young Kim, In-Jun Yeo, Tae-Jun Jung, and Cheon-Jei Kim\*

Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 05029, Korea

### Abstract

The objective of this study was to examine the effect of ginger extract (GE) combined with citric acid on the tenderness of duck breast muscles. Total six marinades were prepared with the combination of citric acid (0 and 0.3 M citric acid) and GE (0, 15, and 30%). Each marinade was sprayed on the surface of duck breasts (15 mL/100 g), and the samples were marinated for 72 h at 4°C. The pH and proteolytic activity of marinades were determined. After 72 h of marination, Warner Bratzler shear force (WBSF), myofibrillar fragmentation index (MFI), pH, cooking loss, moisture content, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and protein solubility were evaluated. There was no significant ( $p>0.05$ ) difference in moisture content or cooking loss among all samples. However, GE marination resulted in a significant ( $p<0.05$ ) decrease in WBSF but a significant ( $p<0.05$ ) increase in pH and MFI. In addition, total protein and myofibrillar protein solubility of GE-marinated duck breast muscles in both WOC (without citric acid) and WC (with citric acid) conditions were significantly ( $p<0.05$ ) increased compared to non-GE-marinated duck breast muscles. SDS-PAGE showed an increase of protein degradation (MHC and actin) in WC condition compared to WOC condition. There was a marked actin reduction in GE-treated samples in WC. The tenderization effect of GE combined with citric acid may be attributed to various mechanisms such as increased MFI and myofibrillar protein solubility.

**Keywords:** ginger extract, citric acid, marination, duck breast muscle, tenderization

Received December 29, 2014; Revised May 12, 2015; Accepted May 17, 2015

### Introduction

Duck muscle is a popular source of meat in Southeast Asia (Li *et al.*, 2013) including China (Liao *et al.*, 2010; Liu *et al.*, 2007) due to its delicate flavor and nutritional value. In South Korea, duck products are increasingly well accepted by consumers. As a high nutritional food, duck meat contains over 70% unsaturated fatty acid (Park *et al.*, 1986). It is rich in essential amino acids such as alanine, valine, glycine, and methionine (Kim and Nam, 1977). However, it is generally tough when compared to other meats (Smith and Fletcher, 1992). For this reason, numerous studies have been conducted to investigate the effect of proteases from natural source on the tenderness of duck meat (Buyukyavuz, 2013; Li *et al.*, 2013; Liu *et al.*, 2007; Tsai *et al.*, 2012).

Nafi *et al.* (2013) and Thompson *et al.* (1973) have

reported that the enzyme activities of ginger extract are affected by pH and that the proteolytic enzyme activity of ginger extract is reduced by decreased pH. On the other side, marination in acidic solutions has been used both traditionally and industrially for the tenderization and flavoring of meat (Berge *et al.*, 2001; Yusop *et al.*, 2010). Abdeldaiem and Hoda (2013) have indicated that “Zingibain” is the main protease associated with the tenderization effect of ginger extract on camel meat. Choi and Laursen (2000) have reported that GP-I and GP-II are the two main proteases in ginger rhizome. GP-I and GP-II have homologies with actinidin, glycyI endopeptidase, omega, and papain (Choi and Laursen, 2000).

Ginger is a typical flavoring agent widely used in the meat industry. According to Tsai *et al.* (2012), ginger extract could improve the tenderness of duck breast muscle. The tenderization effect of ginger extract mainly results from its degradation of myofibrillar proteins such as myosin heavy chain (MHC), troponin T,  $\alpha$ -actinin, and desmin of duck breast muscle (Tsai *et al.*, 2012). Ginger extract also inhibits lipid oxidation of duck breast muscle during marination (Tsai *et al.*, 2012). Several studies have

\*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

been carried out to determine the tenderization effect of ginger extract on other meats (Abdeldaiem and Hoda, 2013; Naveena *et al.*, 2004). Thus, ginger extract could be a useful ingredient to improve the tenderness of duck meat.

The tenderization effect of marination on meat have been examined by using organic acids such as lactic acid (Aktas *et al.*, 2003; Berge *et al.*, 2001; Ertbjerg *et al.*, 1999), citric acid (Aktas *et al.*, 2003; Ke *et al.*, 2009), acetic acid (Chang *et al.*, 2010), and other acidic materials such as soy sauce (Kim *et al.*, 2013), vinegar, wine, and fruit juice (Oreskovich *et al.*, 1992).

Citric acid is often used as a food acidulant for tenderizing meat (Ke, 2006). Ke *et al.* (2009) have reported that beef muscle marinated with citric acid have a more tender texture than the control, with citric acid exhibiting antioxidant effect on beef muscle. However, no information is available about the tenderization effect of citric acid combined with ginger extract on duck breast muscles. Therefore, the objective of this study was to evaluate the effect of different concentrations of ginger extract on the tenderness of duck breast muscle under acidic (citric acid) marination conditions.

## Materials and Methods

### Preparation of ginger extract (GE) and marinade solution

Fresh ginger rhizome (*Zingiber officinalis roscoe*) purchased from a local market was washed, peeled, sliced and immediately homogenized with an equal quantity of chilled and distilled water (4°C) for 2 min to extract the crude enzyme. The homogenate was filtered through two layers of cheese cloth and the water was collected and saved as the ginger extract (GE). Marinades were prepared as 0, 15, and 30% GE. Each marinade with different GE concentration was divided further into two groups, with or without 0.3 M citric acid. Control group of duck breast was prepared without GE (0%) and citric acid.

### Sample preparation

Duck breast muscles (Cherry Valley ducks *musculus pectoralis major*, approximately 43 d old, average live weight 3.34 kg) were purchased from a local market. All subcutaneous and inter-muscular fat and visible connective tissues were removed from the *pectoralis major* muscles, and then sliced into approximately 8 mm thick pieces. All the samples were sprayed with 15% v/w of marinades (15 mL/100 g meat) and were packed in low-density polyethy-

lene bags then marinated for 72 h at 4°C. After 72 h of marination, samples were washed with distilled water by wash bottle, drained and cooked in water bath at 75±1°C for 30 min. The raw meat (before cooking) was evaluated for pH, moisture content, protein solubility and myofibrillar fragmentation index (MFI). Cooked samples were evaluated for cooking loss and Warner Bratzler shear force (WBSF) values.

### pH measurement

The pH values of samples were measured by mixed 5 g raw samples with 20 mL distilled water for 60 s in a homogenizer (Ultra-Turrax SK15, Janke & Kunkel, Germany) and determined with a pH meter (Model 340, Mettler-Toledo GmbH, Switzerland).

### Proteolytic activity assay

The proteolytic activity was determined using the procedure described by Su *et al.* (2009), with slight modifications. 10 mg of azocasein (Sigma-Aldrich) was dissolved in 1 ml of 100 mM MES buffer (pH 6.0) and used as a substrate solution (1%). A 0.5 mL substrate solution was placed into a 1.5 mL micro-centrifuge tube by pipette and 0.5 mL marinade solution was added. The mixture was incubated at 60°C for 20 min and the assay was terminated by added 0.5 mL of 1.5 M HClO<sub>4</sub>. After that, the mixture were mixed thoroughly and stored in ice for 1 h, followed by centrifugation at 15,000 g for 10 min. 0.5 mL of the supernatant fluid was transferred to a 1.5 mL cube and an equal volume of 1 M NaOH was added. Absorbance was measured at 440 nm using a UV/VIS spectrophotometer (Libra S22, Biochrom Ltd., England). 1 mg azo group released per minute was defined as one unit. The proteolytic activity were plotted by a standard curve that obtained by using of different concentrations of azocasein solutions.

### Cooking loss

The cooked meat was cooled at room temperature for 6 h and weighed. The cooking loss values were obtained as follows:

$$\text{Cooking loss (\%)} = \frac{[\text{the weight before cooking (g)} - \text{the weight after cooking (g)}]}{\text{the weight before cooking (g)}} \times 100$$

### Moisture contents

The moisture contents of the marinated raw samples were measured using standard AOAC (2000) methods

and were determined by weight loss after 12 h of drying at 105°C in a drying oven (SW-90D, Sang Woo Scientific Co., Korea).

#### Warner Bratzler shear force (WBSF)

The cooked meats were cooled at room temperature for 6 h. The samples were cut to  $1 \times 0.8 \times 3 \text{ cm}^3$  parallel to the muscle fiber orientation. The WBSF was determined using Warner Bratzler shear attachment (V-type blade set) on a texture analyzer (TA-XT2i, Stable Micro Systems Ltd., England) and test speeds were set at 2 mm/s. The maximum force required to shear through the samples were recorded.

#### Myofibrillar fragmentation index (MFI)

The MFI was determined according to the procedures of Olson and Parrish (1976) and Kim *et al.* (2013) with slight modifications. The myofibrils were extracted by MFI buffer (20 mM  $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ , pH 7.0, 100 mM KCl, 1 mM EDTA, 1 mM  $\text{NaN}_3$ ). The protein concentration of the final suspension was determined by the Biuret method (Gornall *et al.*, 1949) and then diluted with MFI buffer to a protein concentration of  $0.5 \pm 0.05 \text{ mg/mL}$ . The absorbance of the diluted myofibril suspensions were measured at 540 nm using a UV/VIS spectrophotometer (Libra S22, Biochrom Ltd., England) and each absorbance was multiplied by 200 to obtain the MFI values.

#### Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The myofibrillar protein fraction was separated by our MFI method as described. SDS-PAGE was determined according to the method of Kim *et al.* (2013), using 12% running gels and 5% stacking gels (20  $\mu\text{L}$  loaded). The loaded gel was stained with coomassie Brilliant Blue R250 (B7920, Sigma, USA), and then destained in methanol: distilled water: acetic acid (50:40:10). The separated protein bands were identified by comparison with those of standard protein marker (Precision Plus Protein Standards, BioRad Lab., Hercules, USA) and bovine serum albumin (Sigma Chemical Co., USA).

#### Protein solubility

The protein solubility was determined using the procedure described by Helander (1957), with slight modifications. The sarcoplasmic protein solubility was determined by blending 2 g of raw sample and 20 mL of 0.025 M ice-cold potassium phosphate buffer (pH 7.4) in a homogenizer (AM-7, Nihonseiki Kaisha Ltd., Japan). After that, the

mixtures were stored at 4°C overnight and then centrifuged at 6,000 g for 15 min. The supernatants were filtered through a Whatman No.1 filter paper and the filtrate were determined protein concentrations using the biuret method (Gornall *et al.*, 1949). The total protein solubility was determined by blending 2 g of raw sample in 20 mL of ice-cold 1.1 M potassium iodide in 0.1 M potassium phosphate buffer (pH 7.4). The other procedure are described above. The myofibrillar protein solubility was obtained as follows:

$$\text{Myofibrillar protein} = \text{total protein} - \text{sarcoplasmic protein}$$

#### Statistical analysis

A 3 (ginger extract levels)  $\times$  2 (citric acid addition) factorial design with three replicates was employed by using two way analysis of variance (ANOVA). Duncan's multiple range tests ( $p < 0.05$ ) was used to determine differences between treatments means. The significances of difference among treatments (GE level) were subjected to one-way analysis of variance. The significance of difference between WOC (without citric acid) and WC (with citric acid) was determined by t-test by using the SPSS 18.0 for Windows (IBM SPSS Inc., USA).

## Results and Discussion

#### pH values and relative proteolytic activity of marinade solutions

The pH values of different marinade solutions are listed in Table 1. Regardless of being WOC or WC, the pH values of GE added marinade solutions were significantly ( $p < 0.05$ ) higher than that of solutions without GE. The relative proteolytic activity of different marinade solutions are shown in Fig. 1. All WOC marinades showed significantly ( $p < 0.05$ ) higher proteolytic activity than WOC marinades. The proteolytic activity of 15% GE WC treatment was reduced by approximately 78% compared to 30% GE WOC treatment.

#### pH of raw meat

The pH values of raw meat are summarized in Table 2. As expected, the duck breast muscles marinated at WC condition had significantly ( $p < 0.001$ ) lower pH values than those marinated at WOC conditions, regardless of how much GE was added. The lower pH at WC condition might be mainly due to the low pH of citric acid. Ke *et al.* (2009) also found that the pH of beef muscle was decreased by injection of citric acid. As the concentration of

**Table 1. The pH values of marinade solutions**

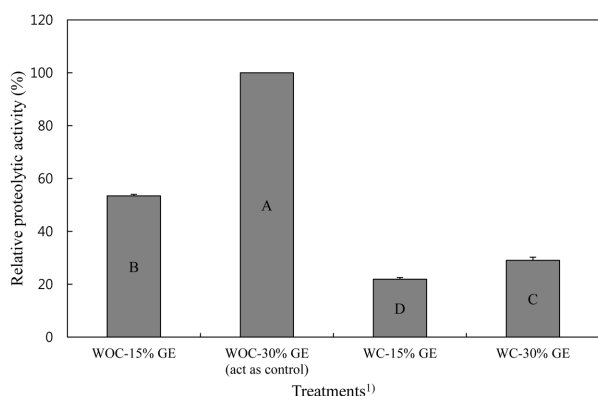
Traits	Treatments <sup>1)</sup>		
	0% GE	15% GE	30% GE
Without citric acid (WOC)	5.47±0.07 <sup>B</sup>	6.02±0.03 <sup>A</sup>	6.02±0.04 <sup>A</sup>
With citric acid (WC)	2.02±0.03 <sup>C</sup>	2.14±0.01 <sup>B</sup>	2.21±0.02 <sup>A</sup>
<i>p</i> -value	***	***	***

All values are mean±SD of three replicates.

<sup>A-C</sup>Means within rows with different superscript letters are significantly different ( $p < 0.05$ ).

<sup>1)</sup>0% GE, marinade solution contain 0% of ginger extract; 15% GE, marinade solution contain 15% of ginger extract; 30% GE, marinade solution contain 30% of ginger extract.

\*Means within columns with different marination conditions (with or without citric acid) are significantly different ( $p < 0.001$ ).

**Fig. 1. Relative proteolytic activity (%) of marinade solutions.**

<sup>A-D</sup>Means different letters between each treatment are significantly different ( $p < 0.05$ ). <sup>1)</sup>Treatments: WOC-15% GE, marinade solution contain 15% of ginger extract without citric acid; WOC-30% GE, marinade solution contain 30% of ginger extract without citric acid; WC-15% GE, marinade solution contain 15% of ginger extract with citric acid; WC-30% GE, marinade solution contain 30% of ginger extract with citric acid.

GE added was increased, pH at both WOC and WC conditions tended to increase. The higher pH of GE-treated samples might be attributed to the relatively high pH of GE. Increased pH of GE-treated buffalo meat has also been reported Naveena *et al.* (2004).

### Cooking loss

Cooking loss is summarized in Table 2. Cooking loss values were not significantly ( $p > 0.05$ ) different from each other between WOC samples and WC samples, regardless of the treatment. According to Naveena *et al.* (2004), the cooking yield of buffalo meat treated with GE has no significant difference compared to the control, although it has been slightly increased. Similarly, Naveena and Mendiratta (2001) have reported that the cooking yield of GE-treated spent hen meat is slightly higher compared to the control without significant difference. Yusop *et al.* (2010)

have indicated that there is no difference in the cooking loss of chicken breast marinated in solutions with various pH containing citric acid. These results might be due to the lack of change in core pH of meats. However, an increase in the cooking yield of citric acid marinated beef has been observed by Ke *et al.* (2009). Although significant differences of pH were found among treatments in this study, there was no significant difference in cooking loss, which might be due to an absence of change in core pH of the muscles.

### Moisture content of raw meat

Moisture content of raw meat is shown in Table 2. The moisture content of marinated duck breast muscles ranged from 74.90% to 75.78%. There was no significant ( $p > 0.05$ ) difference among samples. Naveena *et al.* (2004) have reported that the moisture content of GE marinated buffalo meat is not significantly different from the control samples. Similarly, no difference between the moisture content of raw GE marinated and control camel meat has been observed by Abdeldaiem and Hoda (2013). According to Yusop *et al.* (2010), there is no difference in moisture content of chicken breast marinated in solutions with various pH treated with citric acid. However, Ke *et al.* (2009) have reported that citric acid injected beef muscle has higher moisture content than the control.

### Warner Bratzler shear force

Warner Bratzler shear force (WBSF) is shown in Fig. 2. WBSF is an important indicator related to meat tenderness (Zhao *et al.*, 2012). The WBSF is reduced as the tenderness of meat is improved (Warner 1929; Wheeler *et al.*, 1997). The WBSF values of duck breast muscles marinated in WOC were found to be significantly ( $p < 0.05$ ) reduced as the concentration of GE was increased. The WBSF of the 30% GE sample was reduced by approximately 26% compared to 0% GE sample. These results are in agreement with those of Naveena *et al.* (2004), which has

**Table 2. pH, cooking loss and moisture content of duck breast muscles marinated with ginger extract in WOC (without citric acid) and WC (with citric acid) marinade**

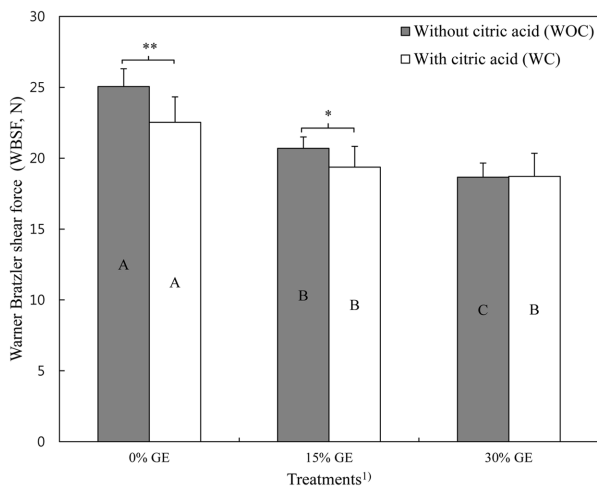
Traits	Treatments <sup>1)</sup>		
	0% GE	15% GE	30% GE
pH (raw meat)			
Without citric acid (WOC)	5.80±0.01 <sup>B</sup>	5.82±0.01 <sup>A</sup>	5.82±0.01 <sup>A</sup>
With citric acid (WC)	4.73±0.02 <sup>C</sup>	4.76±0.03 <sup>B</sup>	4.81±0.00 <sup>A</sup>
<i>p</i> -value	***	***	***
Cooking loss (%)			
Without citric acid (WOC)	40.38±1.06	39.49±0.71	39.44±0.57
With citric acid (WC)	40.10±0.80	39.92±0.69	40.05±0.77
<i>p</i> -value	NS	NS	NS
Moisture content (%)			
Without citric acid (WOC)	75.78±0.91	75.58±0.16	75.34±0.17
With citric acid (WC)	75.00±1.18	74.90±0.99	75.20±0.07
<i>p</i> -value	NS	NS	NS

All values are mean±SD of three replicates.

<sup>A-C</sup>Means within rows with different superscript letters are significantly different ( $p<0.05$ ).

<sup>1)</sup>Treatments: 0% GE, marinade solution contain 0% of ginger extract; 15% GE, marinade solution contain 15% of ginger extract; 30% GE, marinade solution contain 30% of ginger extract.

\*Means within columns with different marination conditions (with or without citric acid) are significantly different ( $p<0.001$ ). NS was no significant difference.

**Fig. 2. Warner Bratzler shear force (WBSF) of duck breast muscles marinated with ginger extract and citric acid.**

<sup>A-C</sup>Means within same marinade condition (WOC or WC) with different letters are significantly different ( $p<0.05$ ).

\*Means within same treatments are significantly different;  $*p<0.05$ .  $**p<0.01$ . <sup>1)</sup>Treatments: 0% GE, marinade solution contain 0% of ginger extract, 15% GE, marinade solution contain 15% of ginger extract, 30% GE, marinade solution contain 30% of ginger extract.

indicated that buffalo meat sprayed with a 5% w/v ginger extract has a lower shear force value than the control. Similar results have been found in studies of Naveena and Mendiratta (2001), Abdeldaiem and Hoda (2013), and Syed Ziauddin *et al.* (1995). Substantial evidence indicates

that the shear force reduction of meat by the treatment with ginger is due to proteolysis of muscle protein such as myofibrillar protein. According to Thompson *et al.* (1973), ginger can degrade collagen. Naveena *et al.* (2004) have reported that ginger can increase the collagen solubility of buffalo meat, which may be the reason why ginger has a tenderization effect on collagen rich muscles. WBSF values of WC samples were significantly ( $p<0.01$ ) lower than those of the WOC sample with 0% GE. This may be caused by the swelling of myofibers due to acidic conditions. According to Berge *et al.* (2001), acidic substances can improve meat tenderness by causing meat fibers to swell. In WC treatments, the WBSF values of GE-treated samples were significantly ( $p<0.05$ ) lower than non-GE-treated samples. The WBSF value of the 30% GE sample was reduced by approximately 17% compared to 0% GE sample. However, there was no significant ( $p>0.05$ ) difference between 15% GE sample and 30% GE sample at WC conditions. According to Thompson *et al.* (1973), the optimum enzyme activity of ginger is from pH 4.5 to pH 6.0 based on bovine serum albumin. This suggests that the protease activity of ginger is optimal in mildly acidic conditions. Nafi *et al.* (2013) have reported that the optimum enzyme activity of ginger is at pH 7.0, with the ability to be active in neutral, mildly acidic, and mildly alkaline conditions. There was no significant difference between the 30% GE sample at WOC condition and that at WC condition. WC treatment could be affected

by proteolysis (due to the remaining ginger enzyme activity and endogenous meat proteases such as cathepsin B, D, and L with optimum activity at acidic conditions) and myofiber swelling (caused by acidic marination). In summary, GE demonstrated a WBSF reduction effect even at the WC condition. However, the range of reduction at WC condition was smaller than that at WOC condition.

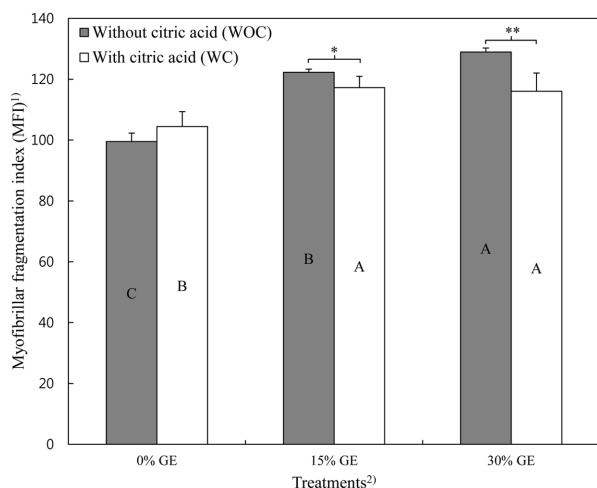
### Myofibrillar fragmentation index

Myofibrillar fragmentation index (MFI) is presented in Fig. 3. MFI is one useful indicator of meat tenderization mainly caused by the proteolysis of myofibrillar protein (Hopkins *et al.*, 2000; Kim *et al.*, 2013; Olson *et al.*, 1976). Breakdowns of Z lines of meat structure will occur during enzymatic tenderization. This can lead to shortening of myofibril length, reduction of sarcomere number, but increase of MFI (Zhao *et al.*, 2012). At WOC conditions, the MFI values were affected by the GE levels in the marinade, with 30% GE demonstrating the highest MFI values among all treatments (an increase of approximately 23% compared to 0% GE,  $p < 0.05$ ). Shin *et al.* (2008) have demonstrated that bovine *longissimus dorsi* muscle treated with protease extract from *Sarcodon aspratus* and papain has significantly higher MFI values than the control. Zhao *et al.* (2012) also have reported that the MFI values of

beef treated with MCP-01 and bromelain has 91.7% and 53.7% increases, respectively. However, the MFI values of beef treated with papain did not exhibit an increase compared to the control. Gerelt *et al.* (2000) have found that meat treated with papain and proteinase from *A. sojae* and *A. oryzae* has significantly higher relative MFI values than that of the control at any stage during storage. At WC condition, the MFI values of GE-treated samples in this study were significantly ( $p < 0.05$ ) higher than those of the control. However, there was no significant ( $p > 0.05$ ) difference between 15% GE and 30% GE. This result is in consistent with that of WBSF. Except for 0% GE, the MFI values of WOC samples were higher than those of WC samples. This might be due to the lower protease activity of ginger at acidic conditions (WC), leading to a reduction of myofibrillar proteolysis. However this results were not in consistent with that of WBSF. This might be caused by the swelling of myofibers and the remaining connective tissue or collagen in WC samples due to acidic condition. Also, this may caused the WC samples to be more or similar extent tender compared to WOC samples.

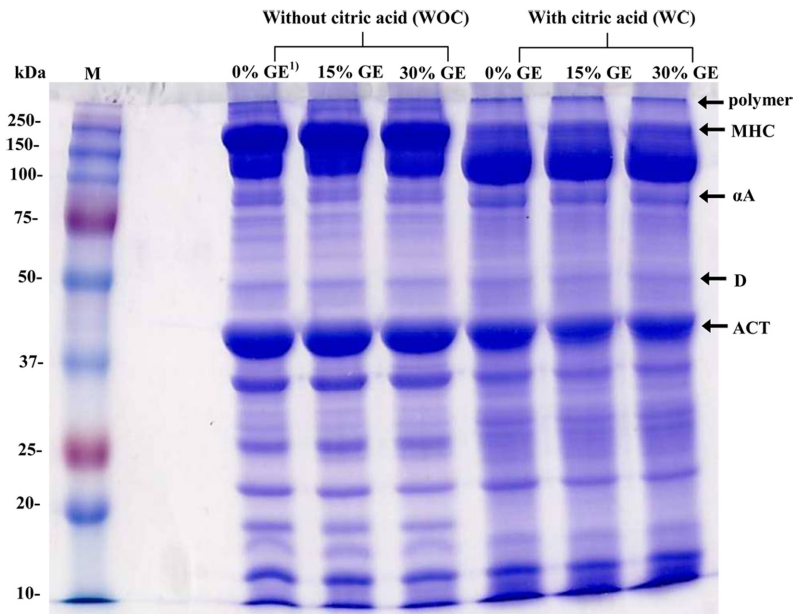
### Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to elucidate the effects of GE combined with citric acid on myofibrillar protein of duck breast muscle (Fig. 4). Our results showed a reduction in the number of protein bands for all WC treatments. This could be considered as an evidence of increased proteolysis of myofibrillar proteins in the WC samples. It has been shown that the degradation of meat proteins is evidenced by a decrease of high molecular weight protein bands and an increase of low molecular weight protein bands (Naveena *et al.*, 2011; Rawdkuen and Benjakul, 2012). The MHC and actin bands in WC samples were markedly degraded when compared to the WOC samples. Moreover, in WC treatment, the actin of GE-treated sample was markedly degraded compared to 0% GE, which correlated with the MFI and WBSF findings. Syed Ziauddin *et al.* (1995) have shown a reduction in the number of protein bands in buffalo muscle treated with lactic acid and ginger at both ambient temperature ( $26 \pm 2^\circ\text{C}$ ) and chilled temperature ( $4 \pm 1^\circ\text{C}$ ). Degradation of MHC, actin, and  $\alpha$ -actinin has been found in lactic acid-injected beef muscle (Berge *et al.*, 2001). It has been reported that several major myofibrillar proteins are degraded in GE-marinated duck breast muscle including titin, MHC, troponin-T, desmin, and  $\alpha$ -actinin, which could partially



**Fig. 3. Myofibrillar fragmentation index (MFI) of duck breast muscles marinated with ginger extract and citric acid.**

<sup>A-C</sup>Means within same marinade condition (WOC or WC) with different letters are significantly different ( $p < 0.05$ ). \*Means within same treatments are significantly different; \* $p < 0.05$ , \*\* $p < 0.01$ . <sup>1)</sup>MFI: Absorbance at 540 nm of 0.5 mg of protein/ml of myofibril solution  $\times 200$ . <sup>2)</sup>Treatments: 0% GE, marinade solution contain 0% of ginger extract, 15% GE, marinade solution contain 15% of ginger extract, 30% GE, marinade solution contain 30% of ginger extract.



**Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) photograph of myofibrils from duck breast muscles marinated with ginger combined with citric acid.** M, standard protein marker; Polymer, probably nebulin and/or titin; MHC, myosin heavy chain;  $\alpha$ A,  $\alpha$ -actinin; D, desmin; ACT, actin. <sup>1)</sup>0% GE, 0% ginger extract.

explain the tenderization effect of GE in GE-treated muscle (Tsai *et al.*, 2012). In this study, WOC treatments displayed no marked difference as the concentration of GE was increased. This might be due to the fact and only a small amount of marinade solution was used in this study compared to that in the study of Tsai *et al.* (2012). These results suggest that the combination of GE and citric

acid can cause higher proteolytic degradation of myofibrillar protein than GE marination of samples alone. The addition of GE might have degraded actin of duck breast muscle even under citric acid conditions. However, there was no marked difference between 15% GE and 30% GE.

**Table 3. Protein solubility (mg/g) of duck breast muscles marinated with ginger extract in WOC (without citric acid) and WC (with citric acid) marinade**

Traits	Treatments <sup>1)</sup>		
	0% GE	15% GE	30% GE
Sarcoplasmic protein (mg/g)			
Without citric acid (WOC)	40.76±0.74 <sup>C</sup>	55.86±1.15 <sup>B</sup>	58.85±0.77 <sup>A</sup>
With citric acid (WC)	39.14±0.76 <sup>B</sup>	40.52±0.03 <sup>A</sup>	37.62±0.22 <sup>C</sup>
<i>p</i> -value	*	***	***
Myofibrillar protein (mg/g)			
Without citric acid (WOC)	119.42±4.87 <sup>B</sup>	140.22±3.31 <sup>A</sup>	151.48±11.02 <sup>A</sup>
With citric acid (WC)	140.98±3.88 <sup>B</sup>	150.05±3.15 <sup>A</sup>	152.57±0.82 <sup>A</sup>
<i>p</i> -value	***	**	NS
Total protein (mg/g)			
Without citric acid (WOC)	160.17±4.36 <sup>C</sup>	196.08±4.43 <sup>B</sup>	210.33±10.37 <sup>A</sup>
With citric acid (WC)	180.12±4.47 <sup>B</sup>	190.57±3.12 <sup>A</sup>	190.19±0.96 <sup>A</sup>
<i>p</i> -value	***	NS	*

All values are mean±SD of three replicates.

<sup>A-C</sup>Means within rows with different superscript letters are significantly different ( $p < 0.05$ ).

<sup>1)</sup>Treatments: 0% GE, marinade solution contain 0% of ginger extract; 15% GE, marinade solution contain 15% of ginger extract; 30% GE, marinade solution contain 30% of ginger extract.

\*Means within columns with different marination conditions (with or without citric acid) are significantly different; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ; NS=no significant difference.

### Protein solubility

Protein solubility of marinated duck breast muscle is shown in Table 3. Regardless of the acid conditions (WOC or WC), significantly higher myofibrillar and total protein solubility values were observed in GE-treated samples compared to 0% GE samples. In addition, all WOC treatments had significantly ( $p < 0.05$ ) higher sarcoplasmic protein solubility than WC treatments. In WOC samples, the sarcoplasmic protein solubility values were significantly ( $p < 0.05$ ) increased as the percentages of GE were increased. Interestingly, at WC conditions, the sarcoplasmic protein solubility of 15% GE was significantly ( $p < 0.05$ ) higher than that of 0% GE. However, the sarcoplasmic protein solubility was significantly ( $p < 0.05$ ) reduced at 30% GE.

Myofibrillar protein solubility values of samples marinated at WOC condition ranged from 119.42 mg/g to 151.48 mg/g. At WC condition, it ranged from 140.98 mg/g to 152.57 mg/g. Regardless of the concentration of GE, myofibrillar protein solubility values at WOC condition were lower than that at WC condition. At both WOC and WC conditions, myofibrillar protein solubility values of 30% GE were higher than those of 15% GE. However, the differences were not statistically significant ( $p > 0.05$ ). Total protein solubility values of samples marinated at WOC condition ranged from 160.17 mg/g to 210.33 mg/g, and those marinated at WC ranged from 180.12 mg/g to 190.57 mg/g. At WOC condition, total protein solubility values were significantly ( $p < 0.05$ ) increased as the concentration of GE was increased. At WC condition, while the inclusion of GE had significantly ( $p < 0.05$ ) higher total protein solubility values than 0% GE, there was no significant ( $p > 0.05$ ) difference between 15% GE and 30% GE.

Increase in protein solubility due to ginger has been reported for buffalo muscle. It has been noted that the increase in permeability of myofibrils could make protein disintegrate easily, which might be the reason why the protein solubility is increased after enzyme treatment (Naveena *et al.*, 2004). Naveena *et al.* (2004) and Abdeldaiem and Hoda (2013) have reported significantly higher myofibrillar and total protein solubility of ginger-treated muscles, with marginal increase in sarcoplasmic protein. Similarly, increase of protein extractability has been reported for 3% GE-treated hen meat in both pre-chilled and post-chilled stage, along with a decrease of shear force as the concentration of GE is increased (Naveena and Mendiratta, 2001). These results indicate that the increased protein extractability may be related to a decrease of the shear

force. Li *et al.* (2013) have indicated that the shear force of cooked duck breast muscle has a highly significant correlation with total, sarcoplasmic, and myofibrillar protein solubility. On the other hand, Shin *et al.* (2008) have reported a reduction in myofibrillar and total protein solubility when treatment is carried out with proteolytic extract from *Sarcodon aspratus*. The results obtained in this study suggested that sarcoplasmic protein solubility might decrease due to the presence of citric acid. However, sarcoplasmic protein solubility maybe not be highly related to the tenderness of duck breast muscles. The solubility of myofibrillar and total protein can increase due to GE treatment even in WC conditions, which may lead to the reduction of WBSF values.

### Conclusion

This study evaluated the tenderization effect of GE in acidic conditions (addition of citric acid) on duck breast muscle. The enzyme activity of GE at WC condition was significantly reduced than that at WOC condition. However, GE combined with citric acid had a significant tenderization effect on duck breast muscle. Shear force values indicated that the WC condition was not worse than the WOC condition. Increased values of MFI and myofibrillar protein solubility were observed in GE-treated samples even at WC condition, which might be the main reason why GE had a tenderization effect on duck breast muscles. Our results indicated that GE had a tenderization effect on duck breast muscle even at WC condition, suggesting that ginger might be a useful tenderizer at weak acidic conditions as a traditional and industrial marination method.

### Acknowledgements

This study was supported by the Brain Korea 21 plus (BK 21+) Project from Ministry of Education (Republic of Korea).

### References

1. Abdeldaiem, M. H. and Hoda, G. M. Ali. (2013) Tenderization of camel meat by using fresh ginger (*Zingiber officinale*) extract. *Food Sci. Qual. Manag.* **21**, 12-25.
2. Aktas, N., Aksu, M. I., and Kaya, M. (2003) The effect of organic acid marination on tenderness, cooking loss and bound water content of beef. *J Muscle Food* **14**, 181-194.
3. AOAC (2000) Official methods of analysis of AOAC. 16th ed, Association of Official Analytical Chemists, Washington



- DC.
4. Berge, P., Ertbjerg, P., Larsen, L. M., Astruc, T., Vignon, X., and Moller, A. J. (2001) Tenderizaion of beef by lactic acid injected at different times post mortem. *Meat Sci.* **57**, 347-357.
  5. Buyukyavuz, A. (2013) Effect of bromelain on duck breast meat tenderization. Master of Science in Food, Nutrition, and Culinary Science, University of Clemson, USA.
  6. Chang, H. J., Wang, Q., Zhou, G. H., Xu, X. L., and Li, C. B. (2010) Influence of weak organic acids and sodium chloride marination on characteristics of connective tissue collagen and textural properties of beef semitendinosus muscle. *J. Texture Stud.* **41**, 279-301.
  7. Choi, K. H. and Laursen, R. A. (2000) Amino-acid sequence and glycan structures of cysteine proteases with proline specificity from ginger rhizome *Zingiber officinale*. *Eur. J. Biochem.* **267**, 1516-1526.
  8. Ertbjerg, P., Mielche, M. M., Larsen, L. M., and Moller, A. J. (1999) Relationship between proteolytic changes and tenderness in prerigor lactic acid marinated beef. *J. Sci. Food Agr.* **79**, 970-978.
  9. Gerelt, B., Ikeuchi, Y., and Suzuki, A. (2000) Meat tenderization by proteolytic enzymes after osmotic dehydration. *Meat Sci.* **56**, 311-318.
  10. Gornall, A. G., Bardawill, C. J., and David, M. M. (1949) Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177**, 751-766.
  11. Helander, E. (1957) On quantitative muscle protein determination. *Acta Physiol. Scand.* **41**, 1-99.
  12. Hopkins, D. L., Littlefield, P. J., and Thompson, J. M. (2000) A research note on factors affecting the determination of myofibrillar fragmentation. *Meat Sci.* **56**, 19-22.
  13. Ke, S., Huang, Y., Decker, E. A., and Hultin, H. O. (2009) Impact of citric acid on the tenderness, microstructure and oxidative stability of beef muscle. *Meat Sci.* **82**, 113-118.
  14. Ke, S. M. (2006) Effect of pH and salts on tenderness and water-holding capacity of muscle foods. Department of Food Science, University of Massachusetts Amherst, USA.
  15. Kim, D. P. and Nam, H. K. (1977) Studies on the duck-meat (1) Amino acid composition of duck-meat protein. *J. Korean Soc. Food Nutr.* **6**, 61-65.
  16. Kim, H. W., Choi, Y. S., Choi, J. H., Kim, H. Y., Lee, M. A., Hwang, K. E., Song, D. H., Lim, Y. B., and Kim, C. J. (2013) Tenderization effect of soy sauce on beef *M. Biceps femoris*. *Food Chem.* **139**, 597-603.
  17. Li, C., Wang, D. Y., Xu, W. M., Gao, F., and Zhou, G. H., (2013) Effect of final cooked temperature on tenderness, protein solubility and microstructure of duck breast muscle. *LWT-Food Sci. Technol.* **51**, 266-274.
  18. Liao, G. Z., Wang, G. Y., Xu, X. L., and Zhou, G. H. (2010) Effect of cooking methods on the formation of heterocyclic aromatic amines in chichi and duck breast. *Meat Sci.* **85**, 149-154.
  19. Liu, Y., Xu, X. L., and Zhou, G. H. (2007) Changes in taste compounds of duck during processing. *Food Chem.* **102**, 22-26.
  20. Nafi, A., Foo, H. L., Jamilah, B., and Ghazali, H. M. (2013) Properties of proteolytic enzyme from ginger (*Zingiber officinale Roscoe*). *Int. Food Res. J.* **20**, 363-368.
  21. Naveena, B. M., Kiran, M., Sudhakar Reddy, K., Ramakrishna, C., Vaithyanathan, S., and Devatkal, S. K. (2011) Effect of ammonium hydroxide on ultrastructure and tenderness of buffalo meat. *Meat Sci.* **88**, 727-732.
  22. Naveena, B. M. and Mendiratta, S. K. (2001) Tenderization of spent hen meat using ginger extract. *Brit. Poultry Sci.* **42**, 344-349.
  23. Naveena, B. M., Mendiratta, S. K., and Anjaneyulu, A. S. R. (2004) Tenderization of buffalo meat using plant proteases from *Cucumis trigonus Roxb* (Kachri) and *Zingiber officinale roscoe* (Ginger rhizome). *Meat Sci.* **68**, 363-369
  24. Olson, D. G. and Parrish Jr, F. C. (1976) Relationship of myofibril fragmentation index to measures of beef steak tenderness. *J. Food Sci.* **42**, 506-509.
  25. Olson, D., Parrish Jr, F. C., and Stromer, M. H. (1976) Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. *J. Food Sci.* **41**, 1036-1041.
  26. Oreskovich, D. C., Bechtel, P. J., Mckeith, F. K., Novakofski, J., and Basgall, E. J. (1992) Marinade pH affects textural properties of beef. *J. Food Sci.* **57**, 305-311.
  27. Park, G. B., Chang, P. H., and Kim, Y. H. (1986) Changes in water holding capacity and fatty acid composition of duck meat during postmortem storage. *J. Inst. Develop. Livestock Prod.* **13**, 1-7.
  28. Rawdkuen, S. and Benjakul, S. (2012) Biochemical and microstructural characteristics of meat samples treated with different plant proteases. *Afr. J. Biotechnol.* **11**, 14088-14095.
  29. Shin, H. G., Choi, Y. M., Kim, H. K., Ryu, Y. C., Lee, S. H., and Kim, B. C. (2008) Tenderization and fragmentation of myofibrillar protein in bovine longissimus dorsi muscle using proteolytic extract from *sarcodon aspratus*. *LWT-Food Sci. Technol.* **41**, 1389-1395.
  30. Smith, D. P. and Fletcher, D. L. (1992) Duckling and chicken processing yield and breast meat tenderness. *Poultry Sci.* **38**, 652-655.
  31. Su, H. P., Huang, M. J., and Wang, H. T. (2009) Characterization of ginger proteases and their potential as a rennin replacement. *J. Sci. Food Agric.* **89**, 1178-1185.
  32. Syed Ziauddin, K., Rao, D. N., and Amla, B. L. (1995) Effect of lactic acid, ginger extract and sodium chloride on electrophoretic pattern of buffalo muscle proteins. *J. Food Sci. Technol.* **32**, 224-226.
  33. Thompson, E. H., Wolf, I. D., and Allen, C. E. (1973) Ginger rhizome: A new source of proteolytic enzyme. *J. Food Sci.* **38**, 652-655.
  34. Tsai, L. L., Yen, N. J., and Chou, R. G. R. (2012) Changes in muscovy duck breast muscle marinated with ginger extract. *Food Chem.* **130**, 316-320.
  35. Warner, K. F. (1929) Progress report of the mechanical test for tenderness of meat. *J. Anim. Sci.* **1929**, 114-116.
  36. Wheeler, T. L., Shackelford, S. D., Johnson, L. P., Miller, M. F., Miller, R. K., and Koohmaraie, M. (1997) A comparison of Warner-Bratzler shear force assessment within and among

- institutions. *J. Anim. Sci.* **75**, 2423-2432.
37. Yusop, S. M., O'Sullivan, M. G., Kerry, J. F., and Kerry, J. P. (2010) Effect of marinating time and low pH on marinade performance and sensory acceptability of poultry meat. *Meat Sci.* **85**, 657-663.
38. Zhao, G. Y., Zhou, M. Y., Zhao, H. L., Chen, X. L., Xie, B. B., Zhang, X. Y., He, H. L., Zhou, B. C., and Zhang, Y. Z. (2012) Tenderization effect of cold-adapted collagenolytic protease MCP-01 on beef meat at low temperature and its mechanism. *Food Chem.* **134**, 1738-1744.