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ARTICLE

Effect of High-pressure Processing on the Quality Characteristics and Shelf-life Stability of Hanwoo Beef Marinated with Various Sauces

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Abstract The effects of high-pressure processing (HPP) treatment on the quality characteristics of low graded Hanwoo beef marinated with five different sauces (soy, fish, fish-soy, soy-fish and fish-soy-meat sauces) obtained from Asian countries were studied. The Hanwoo beef striploins were marinated with the aforementioned sauces (at ratio: 3:1 w/w) for 3 days at 4℃ before they were treated with HPP at 550 MPa for 5 min at 10℃. All the sauces used were equalized to a same salt level of 12.5%, and the samples marinated with a 12.5% brine solution were served as a control. After treating with the HPP, all the samples were stored for further 7 days at 4℃ for analyses. Results revealed that HPP treatments showed some effects on technological quality traits (pH, cooking loss and color) but had no effect on the collagen contents of the marinated beef. Noticeably, the HPP treatment led to the increases in amounts of free amino acids associated with monosodium glutamate–like taste and sweet taste in the samples marinated with fish sauce or soy-fish sauce, fish-soy sauce and fish-soy-meat sauce whereas, it only reduced these amino acids in the samples marinated with soy sauce or control. Furthermore, the total bacteria count in all the marinated beef samples treated with HPP were significantly ($p<0.05$) lower than those of the non-HPP-treated samples throughout the storage periods. It is concluded that HPP could be applied for enhancing the taste-active compounds production such as free amino acids, and improving the shelf-life stability of the marinated beef.

Keywords marination, sauce, beef, amino acid, shelf-life

Introduction

Flavor together with tenderness are among the main attributes determining the palatability of meat and meat products as well as affecting the purchasing decision by consumers (Sitz et al., 2005). Flavor, a sensory impression sensed by taste and smell buds is formed by taste and odor (Mottram, 1994). It is known that the flavor of meat and meat products developed during cooking is the results of the reactions between amino acids and sugars and/or the oxidation and degradation of lipids etc. (Khan et al., 2015; Van Ba et al., 2013). As a part of flavor, meat taste is produced by the taste-active compounds such as; free amino acids (FAAs) etc. (Chen and Ho, 1998; Khan et al., 2015). The FAAs contribute not only to the taste but also the odor development by their participation in the Maillard reaction during the meat is cooked (Spanier et al., 1997; Van Ba et al., 2012). In order to improve the eating quality (e.g., flavor) of meats, a lot of attempts have been made by scientists and researchers through the interventions of pre-and post-harvest factors (Van Ba et al., 2012). Amongst, marination is a widely-used processing method in many meat cuisine cultures in the world. The nature of this method is soaking of meat in seasoned solution mainly containing salt brine, phosphate and species or acids for improving yield, flavor, juiciness, water holding capacity and shelf life stability of meats (Smith and Acton, 2010; Zeng et al., 2016). Furthermore, in many countries the demand for the ready-to-cook marinated meat has considerably increased in recent times (Björkroth, 2005; Sloan, 2010).

In the Asian countries food cultures (e.g., Korea, Japan, China and Vietnam etc.), soy, fish, fish-soy, and fish-soy-meat sauces are among the integral fermented seasonings which are widely used in making meats-based dishes (Nam et al., 2010). Depending on the cuisine cultures of each the country for instance; in Korea, all foods require soy sauce as a main seasoning while, the fish sauce is the most important seasoning for making the meat-based dishes in Southeast Asian countries (Chung et al., 2016). Though these traditional fermented sauces are daily used in many cuisines, however, limited scientific information regarding their utilizations as seasonings in marinated meat products. Additionally, how the marination with these sauces affecting the quality characteristics of the products have not been extensively studied. Till now, few studies used soy sauce in marinade and reported an increased collagen solubility and proteolysis (Kim et al., 2013), and improved palatable taste of marinated meat (Yang et al., 2018).

High-pressure processing (HPP), a non-thermal pasteurization technology, is commercially used mainly in the food industry, especially in the meat processing area to improve the shelf-life stability and quality characteristics of meat products (Wang et al., 2013). The beneficial effects of HPP application is making the meats with high consumer’s acceptance in comparison to other non-thermal decontamination technologies such as ionizing radiation (Bajovic et al., 2012). Applying pressures from 400–600 MPa has been shown to inhibit bacterial activity and pasteurize foods (Smelt, 1998). Recent studies have reported that the HPP treatment could improve tenderness and eating quality of beef and pork (Morton et al., 2017; Sauza et al., 2011).

More to the point, in many meat markets such as; Korea or Japan, the consumers much prefer highly-marbled beef due to its tenderness, more juiciness and flavor rather than the beef with low marbling level (Jo et al., 2012). Thus, the imbalance between the produced and consumed amounts for the low marbling beef may lead to a devaluing and loss of economic profits. Therefore, the suitable solutions (e.g., processing it into added-value products such as marinated meats) for improving the consumer’s acceptance for the low-quality beef are necessary.

To the best of our knowledge, no attention has been paid to the evaluation of the effects of HPP treatment on the quality characteristics of beef marinated with traditional sauces. Thus, the present study aimed at evaluating the effects of HPP on the quality, FAAs and microbiological quality of low quality grade Hanwoo beef marinated with five commonly-used traditional sauces (soy, fish, soy-fish, fish-soy, fish-soy-meat sauces) in Asia.

Materials and Methods

Materials

Beef samples

Beef samples (striploin, *M. longissimus lumborum*) from low quality grade (grade 3) of Hanwoo carcasses (48 months-old Hanwoo cows with cold carcass weight of about 440 kg) were collected at 24 h *post-mortem* from a local slaughterhouse in Naju (Korea).

Traditional sauces

Soy sauce (original salt level 6.27%, pH 4.65), fish-soy sauce (original salt level 8.43%, pH 4.98), fish-soy-meat sauce (original salt level 10.28%, pH 5.20) were purchased from local food companies in Korea. Fish sauce (original salt level 12.50%, pH 5.02) was purchased from Trung Thanh Com. (Ha Noi, Vietnam). Soy-fish sauce (original salt level 8.42%, pH 5.05) was purchased from Kikkoman Com. (Hon Tsuyu, Japan). The fish-soy sauce and soy-fish sauce are different from each other in which the fish-soy sauce (the fish content is more predominant than the soy) and contrast to the soy-fish sauce.

Preparation of marinated beef and high-pressure processing (HPP)

Six different marination treatments of the beef with different sauces were prepared in the present investigation. In order to minimize the unexpected effect caused by the various salt contents in the sauces on the quality changes of the marinated meat, all the sauces were equalized to the same salt concentration (12.5%). The sauce's salt concentration of 12.5% chosen in the present work was referred to those prepared in the commercial marinades available in the markets. A brine solution at the same concentration (12.5%) were used as the control marinade. Furthermore, to determine how the sauces affecting the quality of the products, the common ingredients or herbals were not added. A total of 60 beef steaks with approximately 300±10 g (3-cm in thickness) were prepared from the striploins. For each treatment or control, the steaks (n=10) were randomly taken and placed in polyethylene plastic bags, and then the sauce solutions also were added at a ratio 3:1 (w/w). The bags containing samples were vacuum-packaged immediately, and left for 72 h at 4°C. Thereafter, a half of samples (n=5) from each sauce treatment was treated with the high-pressure at 550 MPa for 5 min at 10°C using a HPP equipment (Hiperbaric, Spain) whereas, the rest of samples not treated with the HPP was served as control (non-HPP). The pressure of 550 MPa used in the present work was referred to that used for meats in the previous studies (Jung et al., 2003; Kruk et al., 2011). After the HPP treatment, all the samples were stored at 4°C for analyses. The samples stored for 7 days were evaluated for the technological quality traits (e.g., pH, color, shear force, cooking loss), FAAs and SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis), while the microbial quality was analyzed on all the samples during the storage (0, 7, 14, and 21 days).

pH measurement

The pH of the samples was measured in duplicates using a portable pH meter (Orion Model 301, Beverly, MA, USA).

Color, cooking loss and Warner-Bratzler shear force (WBSF)

All these analytic parameters were determined on the same sample steaks, in which an approximately 200 g-steak was taken from each sample in the treatments. The color attributes (L*, a* and b*) were firstly measured, followed by cooking loss and WBSF. The colors were determined on the three different freshly cut surfaces of the sample steak via a film lid using a Konica Minolta Spectrophotometer CM-2500d (Minolta, Milton Keynes, UK). The Color was expressed according to the Commission International de l'Eclairage (CIE) system and reported as CIE L* (lightness), CIE a* (redness), and CIE b* (yellowness). After the color measurement, the steak samples were recorded for their initial weights, immediately placed in plastic bags and cooked in a 70℃- preheated water bath until their core temperature had reached 70℃ (Hwang et al., 2004). After cooking, the samples were immediately cooled in 18℃-running water for 30 min. The excess moisture was removed using paper wipers and their final weights were taken. The weights before and after cooking were used to determine the cooking loss. Following the cooking loss measurement, the samples were used for WBSF determination. Each the sample steak was made into six strips that had an average diameter of 0.5 inches and fiber parallel to the longest dimension of at least 2 cm. WBSF values of these six strips of each sample were measured using an Instron Universal Testing Machine (Model 3342; Instron Corporation, Norwood, MA, USA) and the shear force values expressed were expressed as kilograms of force (kgf).

Collagen contents

The total collagen content in the samples was determined by using the colorimetric method of Kolar (1990) with suitable modification. Particularly, a 2 g of samples was hydrolyzed for 16 h with 7N H_2SO_4 at 105℃. The hydrolysate was made up to 500 mL with distilled water and filtered. About 2 mL of the diluted filtrate was taken and added with chloramine T solution in a tube and left for 20 min at room temperature. Thereafter, the 4-dimethylamino benzaldehyde solution was also added, and the mixture was heated at 60℃ for 15 min. The absorbance of samples and hydroxyproline standard was read at 558 nm using a spectrophotometer. A standard calibration curve was plotted for 4-hydroxyproline and regression line drawn. Collagen content was expressed in mg/100 g sample, after converted hydroxyproline into collagen with a conversion factor of 7.14.

For insoluble (or heat stable) collagen content, the homogenized samples in Ringer's solution (Hill, 1966) were heated at 77℃ for 70 min, followed by centrifugation, residual fractions were hydrolyzed in 7N H_2SO_4 for 16 h at 105℃. The hydroxyproline content of the hydrolysate was determined after neutralization, according to the procedure of Kolar (1990). The soluble collagen content was calculated by the differences between the total and insoluble collagen contents.

Free amino acid (FAA) analysis

The samples used for the FAA analysis were taken from the inner area of the sample steaks and the analysis was performed using the method of Dashdorj et al. (2013). Each sample (2.5 g each) was homogenized in 5 mL distilled water, by three strokes of 20 s each at 11,000 × rpm at 4℃ and then centrifuged at 6,619×g for 10 min at 4℃. The FAAs in the extracted samples were analyzed using an LC/MS/MS system (Waters Acquity UPLC/Xevo TQ-S). The chromatographic peaks were separated on a column (50×2 mm column packed with 5 μm particles). Solvent A was ACN: 100 mM ammonium formate (20:80 v/v) and solvent B was ACN: tetrahydrofuran:25 mL ammonium formate: formic acid (9:75:16:0.3 v/v). The separation conditions were set as follows; linear gradient 100% B for 3 min, 83% B for 3.5 min and with 100% A for 3.5 min then maintain 100% B for 7 min and re-equilibrated before next sample injections. The amino acids were identified based on the retention time of the standard amino acid mixture and individual FAA values were expressed as μmol/g of sample

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

In order to determine how the HPP treatment affecting the proteolysis in the products marinated with various sauces, the SDS-PAGE was performed using the method of Laemmli (1970). The protein concentration in the sample was adjusted to 2 mg/mL with SDS-PAGE sample buffer (2× treatment buffer, 2-mercaptoethanol, bromophenol blue; pH 6.8), and then heated to 95℃ for 5 min. The heated samples (15 μL each) were loaded into wells of 4% stacking gel and a 12.5% separating gel using a mini SDS-PAGE electrophoresis system (Bio-Rad). A protein broad range marker (BIO-RAD) was also used.

Microbiological measurement

Total plate counts (TPC) on the samples were determined during storage. Approximately 10 g of each sample were aseptically taken and placed in sterile stomacher bag containing 90 mL of peptone water. After being homogenized for 1 min, serial dilutions were made using the peptone water. About 1 mL of the diluted sample was plated on a petrifilm (Aerobic Count Plate, 3M Health Care., Paul, MN, USA). The plates were incubated at 37°C for 48 h in an incubator. Each sample was done in duplicates and TPC was expressed as log number of colony forming unit per gram sample (Log CFU/g).

Statistical analysis

Data were analyzed as a completely randomized deign with 6×2 factorial arrangement using the GLM procedure of SAS Version 9.3 (SAS Institute, Cary, NC, USA) considering the HPP treatment and marination as the main effects, except the FAA data which only considered the HPP treatment as the main effect excluded marination. The significant differences ($p<0.05$) between means was tested by Duncan's multiple range tests.

Results and Discussions

Technological quality traits

The effect of HPP treatment on the technological quality characteristics (pH, shear force and cooking loss) of low graded Hanwoo striploin samples marinated with various sauces after 7 days storage is present in Table 1. According to pH, no statistical differences in mean values occurred among the sauce treatments ($p>0.05$), whereas it was observed that the HPP treatment caused significant increases in the values for all the samples in comparison to the non-HPP treated samples. However, the increasing level of pH differed depending on the kinds of sauces used for instance; samples marinated with soy-fish sauce showed the highest increasing rate (by 0.39 unit) while those marinated with fish-soy-meat sauce only increased by 0.05 unit. In general, the pH values observed in all the marinated samples subjected to the non-HPP or HPP treatment were general lower than their initial values (fresh striploin, pH=5.67) before marination. These declines in pH values after marination with sauces, vacuum-packaging and storage could be related to the fermenting activity by lactic acid bacteria resulting in organic acids production. pH is among the important factors affecting the shelf-life of foods; the lower the pH, the

Table 1. Effect of high-pressure processing on technological quality traits of Hanwoo beef marinated with different sauces at 7-day storage

Traits	Treatment	Marination						SEM
		Brine solution	Soy sauce	Fish sauce	Soy-fish sauce	Fish-soy sauce	Fish- soy- meat sauce	
pH	Non-HPP	5.22^{b}	5.25^{b}	5.23^{b}	5.30^{b}	5.27^{b}	5.34^{b}	0.04
	HPP	5.53^{a}	5.57^{a}	5.39^{a}	5.69^{a}	5.40^{a}	5.39^{a}	1.23
WBSF (kgf)	Non-HPP	2.90^{bC}	2.99^{bBC}	5.03^{A}	2.33^{C}	2.72^{bC}	3.47^{bB}	0.95
	HPP	5.22^{aA}	5.17^{aA}	4.81^{AB}	3.25^{C}	4.46^{aB}	4.73^{aAB}	0.72
Cooking loss (%)	Non-HPP	20.90^{bA}	19.50^{aA}	21.30^{aA}	9.40^{bC}	17.09^{bAB}	18.60^{bAB}	4.39
	HPP	25.20^{aA}	17.50^{bBC}	18.90^{bAB}	12.30^{aC}	23.00^{aAB}	21.50^{aB}	4.57

Means within each column with different letters ($^{a,\,b}$) are significantly different ($p<0.05$).
Means within each row with different letters ($^{A–C}$) are significantly different ($p<0.05$).
HPP, high-pressure processing; WBSF, Warner-Bratzler shear force.

higher the shelf-life stability. In the present study, though the HPP treatment caused the pH increases for all the treated samples, however, these values were still lower than those reported for fresh beef muscles from the same cattle breed in literature (Park et al., 2015). Similar to our finding, previous studies treated fresh beef or pork muscles with HPP and reported an increase in ultimate pH values (Morton et al., 2017; Souza et al., 2011).

One of the benefits obtained from marination of the low graded Hanwoo striploin with the sauces in the present work was the tenderization since the shear force values for all the marinated samples were much reduced as compared to their initial values (6.56 kgf) at 24 h *post-mortem* before marinating. The striploin samples of Hanwoo beef from low quality graded carcasses (grade 3) has been reported to have the shear force values of about 7.89 kgf after 4 days ageing (Yim et al., 2015). We observed that the HPP treatment and type of sauces both significantly affected the shear force values of the marinated beef. Regarding the sauce's effects on the non-HPP treatment, the shear force value was lowest in the samples marinated with soy-fish sauce, followed by fish-soy sauce and the highest was found in the fish soy marinated samples. On the pressurized samples, the lowest shear force value was also found for the samples marinated with soy-fish sauce ($p<0.05$). Based on these shear force results it may be suggested that marination with fish-soy or soy-fish sauces produced the faster tenderization rate in comparison to the other remaining sauces or brine solution. Regarding the WBSF decreases observed for the marinated beef samples in the present work, despite we cannot yet offer a satisfactory explanation for this phenomenon, however, it may be related to the muscle proteolytic activity in the muscle enhanced by the sauces or the presences of endogenous proteases in sauces (Gildberg and Xian-Quan, 1994).

Regarding the HPP treatment, except the samples marinated with the fish sauce or soy-fish sauce, all other remaining samples increased in shear force values (about 0.9–2.2 kgf) as the HPP was applied ($p<0.05$). Though the HPP has been used in meat industry to improve meat tenderness (Morton et al., 2017), however, the tenderization efficacy of this technique has been reported to require high temperature and pressure (Bouton et al., 1977). Under the current study's experimental condition, the samples were treated with HPP at low temperature (10℃). Similar to our results, an increase in WBSF value was reported for pork meat after being treated with HPP (Souza et al., 2011). Our study for the first time used the HPP technique for the low graded beef marinated with various sauces, the HPP seemed not to affect the tenderization process of the samples marinated with fish sauce or soy-fish sauce. However, the samples marinated with the other sauces such as; soy sauce or fish-soy-meat sauce, the tenderization seemed to be delayed but not inhibited because we saw that the WBSF values in these marinated samples continued to decline after 14 or 21 days of storage (data not shown).

The HPP treatment and type of sauce used also significantly affected the cooking loss of the marinated products. For both non-HPP and HPP treatment, the samples marinated with soy-fish sauce presented the lowest (9.40%–12.30%) whereas, those marinated with brine solution (control) showed the highest cooking loss level (20.90%–25.20%). The HPP application resulted in decreases in cooking loss of samples marinated with soy sauce or fish sauce but it increased in the samples marinated with the other remaining sauces. Similar to our finding, Morton et al. (2017) treated fresh beef with HPP at 250 MPa and reported a decrease in cooking loss. A study on pork by Souza et al. (2011) reported that applying HPP at 215 MPa resulted in a decrease in cooking loss of *longissimus* muscle but an increase in cooking loss of *triceps brachii* was observed. From our results, it may be said that the effect of HPP on cooking loss of the marinated beef varied depending on the types of sauces used. And the results indicating the cooking loss differences could be attributed to the differences in denatured protein levels among the sauce treatments.

Effect on collagen content

The collagen contents in Hanwoo beef marinated with different sauces at 7-day storage are presented in Table 2. It was

Table 2. Effect of high-pressure processing on collagen content (g/100 g) of Hanwoo beef marinated with different sauces 7-day storage

Traits	Treatment	Marination						SEM
		Brine solution	Soy sauce	Fish sauce	Soy-fish sauce	Fish-soy sauce	Fish- soy- meat sauce	
Total collagen	Non-HPP	0.26^{C}	0.35^{B}	0.34^{AB}	0.30^{BC}	0.32^{AB}	0.42^{A}	0.05
	HPP	0.23	0.32	0.29	0.30	0.40	0.24	0.06
Insoluble collagen	Non-HPP	0.12	0.23	0.26	0.14	0.16	0.20	0.05
	HPP	0.14	0.15	0.13	0.21	0.40	0.14	0.01
Soluble collagen	Non-HPP	0.15	0.12	0.07	0.16	0.16	0.21^{a}	0.04
	HPP	0.10	0.17	0.16	0.15	0.12	0.10^{b}	0.03

Means within each column with different letters ($^{a,\ b}$) are significantly different ($p<0.05$).
Means within each row with different letters ($^{A–C}$) are significantly different ($p<0.05$).
HPP, high-pressure processing.

reported that the HPP treatment causes proteins denaturation (Jung et al., 2000). In order to determine whether the HPP treatment affects the collagen, the total collagen, soluble and insoluble collagen contents were analyzed and the results showed that the HPP treatment had minor influences on these contents. The use of HPP did not significantly ($p>0.05$) alter the total collagen or soluble and insoluble collagen in all the marinated samples, except the samples marinated with fish-soy-meat sauce which showed a decrease in soluble collagen. We observed that the type of sauces used affected the total collagen content in which all the samples marinated with the sauces had higher level as compared to the ones marinated with brine solution (control) ($p<0.05$). This could be related to either the variations in the collagen content present in the sauces or the increased collagen degradation caused by these sauces. Our results support the finding of Souza et al. (2011), who reported that HPP treatment did not affect the total collagen or soluble and insoluble collagen contents in meat.

Effect on color attributes

The effects of HPP treatment and sauce types on the color traits of the marinated beef after 7 days storage are present in Table 3. The HPP treatment led to the significant increases in L*- and b*-values for all the marinated samples ($p<0.05$). Whereas, the a*-values were significantly decreased in all the samples (except the samples marinated with brine solution)

Table 3. Effect of high-pressure processing on the color traits of beef marinated with different sauces 7-day storage

Traits	Treatment	Marination						SEM
		Brine solution	Soy sauce	Fish sauce	Soy-fish sauce	Fish-soy- sauce	Fish-soy- meat sauce	
CIE L* (Lightness)	Non-HPP	35.0^{bA}	33.1^{bAB}	31.6^{bB}	33.6^{bAB}	33.0^{bAB}	31.2^{bB}	1.38
	HPP	46.8^{a}	49.8^{a}	46.2^{a}	49.4^{a}	45.7^{a}	43.4^{a}	2.40
CIE a* (Redness)	Non-HPP	11.2^{C}	14.5^{AB}	12.7^{aB}	15.6^{aA}	14.9^{aA}	12.4^{aB}	1.70
	HPP	10.7	13.6	7.85^{b}	8.60^{b}	13.0^{b}	10.6^{b}	2.29
CIE b* (Yellowness)	Non-HPP	10.4^{b}	11.2^{b}	9.7^{b}	12.1	11.5^{b}	10.2^{b}	0.98
	HPP	12.4^{a}	16.2^{a}	11.6^{a}	14.4	15.2^{a}	12.2^{a}	1.86

Means within each column with different letters (^{a,b}) are significantly different ($p<0.05$).
Means within each row with different letters ($^{A–C}$) are significantly different ($p<0.05$).
HPP, high-pressure processing.

when the HPP was applied ($p<0.05$). Till now, no data reported on the effect of HPP treatment on color of marinated meat is available, however, similar to our observations other studies on fresh meat have shown that the application of HPP caused a decrease in redness and an increase in lightness (Souza et al., 2011). The changes in color could be resulted from the high pressure which changed the myoglobin content (denaturation) in the products (Carlez et al., 1995) or the changes in sauce's color itself after the HPP treatment. Additionally, when compared to the lightness and redness degrees of the samples marinated with brine solution (control) without HPP treatment, those marinated with all the other sauces (e.g., soy, fish sauce etc.) were redder in color ($p<0.05$). These color changes could result from the suppression of the added sauce's color. A study by Zhuang and Bowker (2016) also reported that marinating chicken breast with sodium chloride and tripolyphosphate reduced the light color of the product. These authors also reported a reduction in red color of the products as marinated with the marinating solutions. From the obtained results it may be said that the marination of low graded striploins with these traditional sauces could improve the attractive colors of the products as compared to the use of brine solution (control).

Effect on free amino acids

The concentrations (μmol/g) of FAAs detected on the beef samples marinated with various sauces and treated with HPP after 7 days storage are presented in Table 4. Till now, there are over hundreds of different amino acids which have been identified however only twenty among them are important for protein synthesis (Wu, 2009). According to the physiological functions, eight (isoleucine, leucine, lysine, methionine, tryptophan, threonine, valine and phenylalanine) are considered as essential amino acids because the body cannot make and must be supplemented through diet. In the present study, a total of 19 FAA was detected, interestingly all of the aforementioned essential amino acids were also found from the marinated samples. Based on the contributions to the tastes development and the similar taste qualities, these amino acids could be

Table 4. Effect of high-pressure processing on free amino acid contents(μmol/g) of Hanwoo beef marinated with different sauces at 7-day storage

Amino acid	Treatment	Marination						SEM
		Brine solution	Soy sauce	Fish sauce	Soy-fish sauce	Fish-soy sauce	Fish-soy- meat sauce	
Monosodium glutamate – like taste								
Aspartic acid	Non-HPP	0.3	5.2[a]	10.3	1.5[b]	11.1[b]	10.9[b]	0.49
	HPP	0.3	3.2[b]	10.3	3.8[a]	10.4[a]	13.3[a]	0.51
Glutamic acid	Non-HPP	1.1[a]	9.0[a]	33.3[b]	24.9[b]	37.6	25.6[b]	4.11
	HPP	0.5[b]	6.1[b]	40.0[a]	46.6[a]	39.6	29.2[a]	1.92
Glutamine	Non-HPP	4.1	8.8[a]	6.0[b]	5.6[b]	4.4[b]	5.6[a]	1.67
	HPP	4.7	5.0[b]	6.8[a]	8.5[a]	4.7[a]	3.8[b]	0.73
Asparagine	Non-HPP	1.2	3.3[a]	1.2	1.5	0.6	1.5	0.19
	HPP	1.2	2.4[b]	1.2	1.5	0.6	1.5	0.58
Sweet taste								
Glycine	Non-HPP	1.9	4.0	26.6[b]	1.7[b]	21.6	11.2[b]	1.07
	HPP	nd	3.6	28.6[a]	4.7[a]	21.7	17.4[a]	1.15
Threonine	Non-HPP	0.8[a]	6.5[a]	12.0[b]	2.8[b]	15.9[b]	11.9[b]	5.90
	HPP	0.5[b]	4.5[b]	16.5[a]	3.7[a]	16.5[a]	15.1[a]	0.33

Table 4. Effect of high-pressure processing on free amino acid contents(μmol/g) of Hanwoo beef marinated with different sauces at 7-day storage (continued)

Amino acid	Treatment	Marination						SEM
		Brine solution	Soy sauce	Fish sauce	Soy-fish sauce	Fish-soy sauce	Fish-soy- meat sauce	
Alanine	Non-HPP	9.0[b]	23.7[a]	31.0[b]	16.0[b]	67.3[b]	35.0[b]	2.04
	HPP	9.7[a]	20.3[b]	37.6[a]	14.3[a]	68.6[a]	44.6[a]	2.22
Serine	Non-HPP	1.1[a]	8.8[a]	10.3[b]	4.4[b]	16.7	12.6[b]	5.61
	HPP	0.7[b]	6.2[b]	13.0[a]	6.3[a]	16.9	15.9[a]	0.64
Proline	Non-HPP	0.6	9.2[a]	7.9[b]	3.6[b]	21.3[b]	11.8[b]	7.21
	HPP	0.6	6.8[b]	10.2[a]	5.7[a]	22.4[a]	15.1[a]	0.70
Bitter taste								
Valine	Non-HPP	2.0[a]	10.0[a]	18.7[b]	5.1[b]	31.9[b]	17.6[b]	1.09
	HPP	1.3[b]	7.5[b]	26.0[a]	6.6[a]	33.7[a]	24.3[a]	1.30
Histidine	Non-HPP	0.6	1.5	6.4[b]	1.4[b]	8.1	5.0[b]	0.43
	HPP	0.6	1.4	8.3[a]	2.5[a]	8.0	6.8[a]	0.48
Leucine	Non-HPP	3.1[a]	13.2[a]	18.8[b]	6.7[b]	24.7[b]	14.0[b]	7.85
	HPP	1.9[b]	9.2[b]	23.3[a]	8.8[a]	26.8[a]	19.1[a]	9.67
Isoleucine	Non-HPP	1.5[a]	8.6[a]	13.3[b]	4.0[b]	18.2[b]	9.9[b]	6.08
	HPP	1.0[b]	6.0[b]	16.9[a]	5.5[a]	20.1[a]	14.0[a]	7.49
Arginine	Non-HPP	1.3	3.0	1.7[b]	1.8[b]	2.8[b]	4.0[b]	1.01
	HPP	0.9	3.2	2.4[a]	3.7[a]	3.3[a]	5.2[a]	1.42
Tryptophan	Non-HPP	nd	0.4[a]	7.4[b]	0.4	3.7[a]	4.1	1.92
	HPP	nd	0.2[b]	7.8[a]	nd	2.9[b]	4.1	2.42
Lysine	Non-HPP	1.8[a]	6.4[a]	27.4[b]	3.9[b]	73.7[b]	30.4[b]	3.25
	HPP	1.5[b]	5.7[b]	48.8[a]	5.7[a]	80.2[a]	43.5[a]	2.78
Tyrosine	Non-HPP	1.1	1.7[a]	2.1[b]	1.2	3.7	2.4	3.17
	HPP	0.7	1.4[b]	2.3[a]	0.5	3.3	2.2	1.21
Phenylalanine	Non-HPP	1.6	4.9[a]	10.2[b]	3.1[b]	13.4	8.6[b]	1.17
	HPP	1.1	3.5[b]	11.7[a]	4.2[a]	13.5	10.4[a]	0.45
Sulfur note								
Methionine	Non-HPP	0.7[a]	2.1[a]	6.3[b]	1.0[b]	8.9[b]	4.4[b]	0.22
	HPP	0.3[b]	1.5[b]	8.7[a]	1.3[a]	9.5[a]	6.2[a]	0.31

Means within each column with different letters ([a, b]) are significantly different ($p<0.05$).
Means within each row with different letters ([A–C]) are significantly different ($p<0.05$).
HPP, high-pressure processing; nd, not detectable.

divided into several classes such as; Monosodium glutamate like taste (MSG-like: including acidic and amidic amino acids), sweet taste (alanine, glycine, serine, threonine and proline), bitter taste (arginine, lysine, phenylalanine, tyrosine, isoleucine, leucine and valine), and sulfuric note (cysteine a methionine) (Dashmaa et al., 2013; Koga et al., 1987; Nishimura, 1998).

The HPP treatment significantly affected the amounts of the amino acids associated with the MSG-like taste, however, the effects differed depending on the type of sauces used. For instance, the HPP treatment resulted in increases in amounts of most of these amino acids in all the samples marinated with fish sauce or soy-fish sauce, fish-soy sauce and fish-soy-meat sauce whereas, it reduced the amount of MSG-like taste amino acids in the samples marinated with the soy sauce and control. Among these amino acid, glutamic acid has been used as an additive in food processing to improve the umami taste (Fuke, 1994).

Regarding the sweet taste-associated amino acids, results revealed that the HPP treatment also significantly ($p<0.05$) reduced the levels of all these amino acids in the samples marinated with soy sauce. Interestingly, the concentrations of all the sweet tasted amino acids significantly ($p<0.05$) increased in the samples marinated with the other remaining sauces (fish sauce, soy-fish sauce, fish-soy sauce and fish-soy-meat sauce). The HPP treatment also caused the increases in amounts of some bitter taste-associated amino acids in all samples marinated with the sauces, except the ones marinated with soy sauce. However, it was also observed that the HPP treatment did not affect the levels of some bitter taste-associated amino acids (e.g., tyrosine, phenylalanine or tryptophan) on some treated samples. Though the increases in amounts of some bitter taste-associated amino acids in the marinated samples treated with the HPP were observed, however, it should be known that most of these amino acids are flavor precursors whose amounts may be reduced due to their participation in the Maillard reactions to produce aroma compounds during cooking (Mottram, 1994). The results indicating the increased FAA contents in marinated samples caused by the HPP treatment could be attributed to enhanced endogenous proteolytic activity due to the release of protease from lysosomes in the beef samples (Ohmori et al., 1991). Similar to our results, Yang et al. (2018) also reported that the HPP treatment of marinated pork resulted in increased FAAs in the products. Thus, it may be said that the application of HPP produced some beneficial effects on the production of taste-active compounds like FAAs in the low graded Hanwoo striploins marinated with fish sauce or soy-fish sauce, fish-soy sauce and fish-soy-meat sauce.

As an important evidence indicating the enhanced endogenous proteolytic activity resulting in the increased FAA in the marinated samples as mentioned above, the SDS-PAGE of the proteins extracted from the same samples at the same storage time

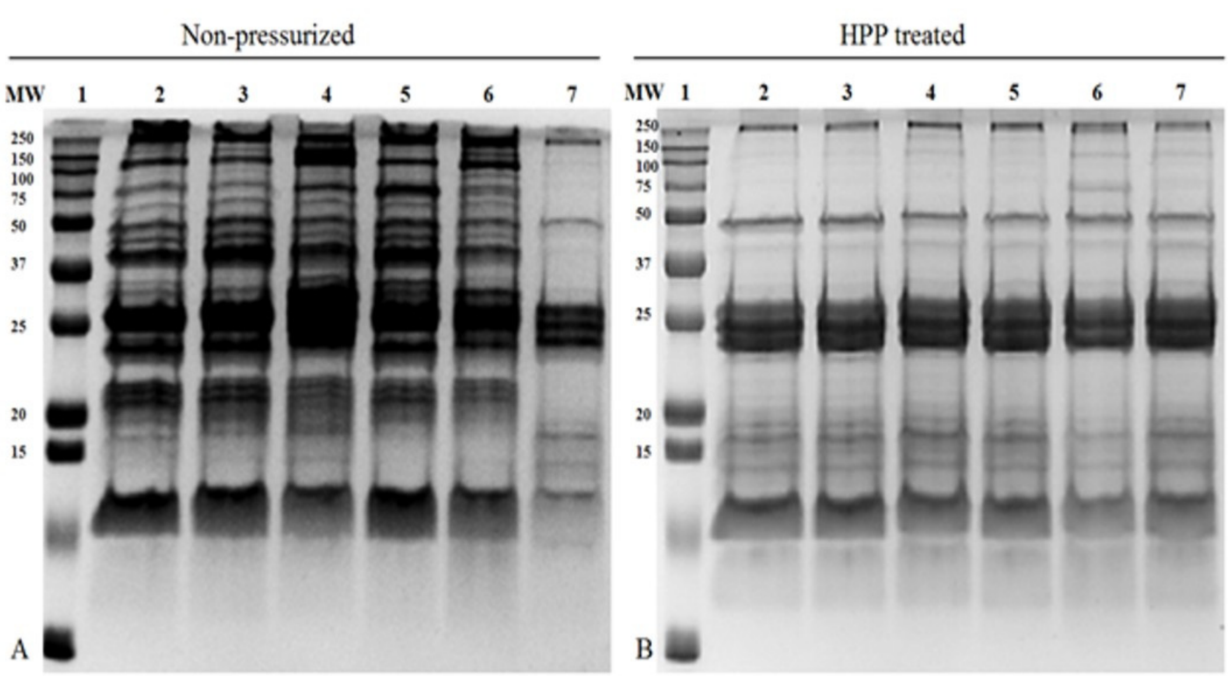

Fig. 1. SDS-PAGE protein degradation patterns of marinated samples treated with Non-HPP (A) and HPP (B) at 7-day storage. Line 1=protein marker, lines 2–7: beef samples marinated with brine solution, soy sauce, fish sauce, soy-fish sauce, fish-soy sauce and fish-soy-meat sauce, respectively. SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; HPP, high-pressure processing.

(7 day) were done and the results are presented in Fig. 1. It shows that the bands with larger molecular weights (35–250 kDa) were denser in the non-HPP treated samples (control) rather than in the samples treated with HPP. This could be related to increased protein degradation or solubilization of sarcoplasmic proteins due to protein denaturation (Marcos et al., 2010). While, bands in the range of 16 kDa increased in intensity for the samples where the HPP treatment was applied, especially in the samples marinated with fish sauce, soy-fish sauce and fish-soy-meat sauce. This phenomenon could be either due to the solubilization of myofibrillar proteins or accumulation of degraded protein products. Additionally, the variations in the intensities of the particular protein bands in the samples could be related to the effects of type of sauces which caused different proteins degradation patterns. Thus, it may be said that the results of SDS-PAGE could be an important evidence explaining for the increases of FAAs contents in the marinated beef samples after treating with the HPP.

Effect on total plate count (TPC)

The effect of HPP treatment on the TPCs in the beef samples marinated with different sauces during storage (1–21 days) is shown in Table 5. Results showed that the samples treated with HPP presented significantly lower TPC in comparison with the non-HPP control on all the storage days examined ($p<0.05$). For instance, at 1-day storage, the TPC ranged among the sauce treatments without HPP treatment from 3.31–3.67 (Log CFU/g) while, the samples treated with HPP showed significantly lower TPC (2.14–2.88 Log CFU/g). The similar trend was kept constantly over the storage up to day 21, at this period (day 21) the non-HPP treated samples (control) presented significantly higher TPC (4.91–6.28 Log CFU/g) than the HPP-treated samples (3.99–5.19 Log CFU/g) ($p<0.05$). No significant differences in TPC occurred among the samples marinated with different sauces among the storages, which may be attributed to the same salt level (12.5%) in all the sauces used ($p>0.05$). Similar to our results, previous studies have also reported that the HPP treatment at 500–600 MPa resulted in delay of microbial growth in fresh beef (Jung et al., 2003) or inhibition of major bacteria species in vacuum-packed cooked ham (Han et al., 2011). On chicken fillets, Kruk et al. (2011) reported that treating these meat samples with HPP at 450–600 MPa significantly reduced bacteria counts in the samples during storage. Smelt (1998) proposed the action mode of high pressure on bacterial inactivation as follows: the increased hydrostatic pressure produces some negative effect on gene expression and protein synthesis, so that the HPP treatment affects the DNA replication of microorganisms, as rising the

Table 5. Effect of high-pressure processing on total plate count (Log CFU/g) in beef samples marinated with different sauces during storage at 4℃

Variables	1 d		7 d		14 d		21 d	
	Non-HPP	HPP	Non-HPP	HPP	Non-HPP	HPP	Non-HPP	HPP
Brine solution	3.43[a]	2.20[b]	3.54[a]	2.32[b]	5.07[a]	3.93[b]	6.28[a]	4.37[b]
Soy sauce	3.31[a]	2.66[b]	3.46[a]	2.99[b]	3.84[a]	3.05[b]	5.42[a]	3.99[b]
Fish sauce	3.54[a]	2.45[b]	3.78[a]	2.88[b]	3.94[a]	3.07[b]	5.19[a]	4.29[b]
Soy-fish sauce	3.33[a]	2.14[b]	3.51[a]	2.45[b]	3.63[a]	2.90[b]	4.91[a]	4.30[b]
Fish-soy sauce	3.67[a]	2.75[b]	4.01[a]	2.89[b]	4.66[a]	4.79[b]	5.63[a]	5.19[b]
Fish-soy-meat sauce	3.56[a]	2.88[b]	3.81[a]	2.98[b]	4.43[a]	4.73[b]	5.21[a]	5.04[b]
SEM	0.14	0.30	0.22	0.29	0.55	0.87	0.48	0.47

Means within each column with different letters ([a,b]) are significantly different ($p<0.05$).
HPP, high-pressure processing.

pressure from 400–600 MPa further changes may be occurred in the mitochondria and cytoplasm. Thus, it may be said that the application of HPP may be considered as an ideal intervention for improving the microbiological quality of the marinated meat products.

In conclusion, the present work, the low-graded Hanwoo striploins were used for marinating with five common traditional sauces available in Asia and the marinated beef samples were also treated with high pressure at 550 MPa before stored at 4℃ for analyses. The types of sauces used considerably affected the tenderization process (indicated by shear force), faster rate in samples marinated with fish-soy sauce after 7 days storage. The HPP treatment did not inhibit the tenderization process but it may delay the tenderization rate in the meat samples marinated with soy sauce or soy-fish sauce. The HPP treatments produced beneficial effects on production of FAAs which may contributes to the tastes developments of the products during cooking. Furthermore, the microbial quality of the marinated beef samples was considerably improved following the HPP treatment at 550 MPa for 5 min at 10℃.

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