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Improved Oxidative Stability of Enhanced Pork Loins Using Red Perilla Extract

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Abstract

Enhanced meat is defined as fresh meat that has been minimally processed to improve quality and consistency. The present work investigated the quality of enhanced pork loins manufactured with the extract of red perilla leaves (ERP). ERP was prepared by the aqueous extraction of red perilla leaves followed by lyophilization. Enhanced pork loins were produced by injecting brine (15% v/w). The treatments consisted of a control (brine containing no ERP), ERP 0.2 (brine containing ERP at a concentration of 2 g/kg of pork loin), and ERP 0.4 (brine containing ERP at a concentration of 4 g/kg of pork loin). The enhanced pork loins were stored at 4°C for 7 d, and its quality parameters were investigated. Addition of ERP decreased the *L**-value and increased the *a**- and *b**-values of enhanced pork loins compared to those of the control group at all storage intervals ($p < 0.05$). A significantly lower pH than that of the control was found in ERP 0.4 after 7 d of storage. The malondialdehyde contents of the cooked pork loins were significantly lower in ERP 0.2 and ERP 0.4 than in those of the control after 4 and 7 d of storage ($p < 0.05$). While ERP 0.4 received relatively low scores in taste, flavor, and overall acceptability of the cooked pork loins ($p < 0.05$), no significant differences were found between the control and ERP 0.2. Enhanced pork loins can be produced using ERP to improve their oxidative stability.

Keywords enhanced meat, oxidative stability, natural antioxidant, red perilla

Introduction

Quality is the major factor that influences consumers' choice of meat. Various methods such as packaging and processing are generally used to maintain and enhance the quality of meat (Kim *et al.*, 2006; Wilkinson *et al.*, 2006). The interest in enhanced meat has recently increased. Enhanced meat is a category of processed meats. However, it is called enhanced or injected fresh meat (USDA, 2005). Enhanced meat is manufactured by the injection of brine containing ingredients and additives in a minimal process to improve quality (Hayes *et al.*, 2006). Salt and phosphate, especially alkaline phosphates, are generally used to improve palatability of enhanced meat (Hoffman, 2003). Furthermore, organic acids and antioxidants can be used in enhanced meat for improvement of shelf life (Djenane *et al.*, 2003; Jensen *et al.*, 2002).

Lipid oxidation is one of major reasons for the deterioration of quality and

decline in shelf life of meat, with changes in flavor, color, texture, odor, and nutritional value (Khan *et al.*, 2016; Georgantelis *et al.*, 2007). Polyunsaturated fatty acids are particularly susceptible to lipid oxidation because the carbon-hydrogen bond adjacent to the double bond is weak and consequently, the hydrogen is easily abstracted to form a free radical (Nimse and Pal, 2015). Synthetic or natural antioxidants can be used for inhibition of lipid oxidation. However, the use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has been excluded from the meat industry because of consumer concerns about toxic substances derived from synthetic antioxidants. Therefore, the interest in antioxidants derived from natural substances has increased, and researchers have searched for natural antioxidants possessing high activity (Mugisha *et al.*, 2016; Zhang *et al.*, 2016). There are various antioxidants that can be derived from natural substances; Vitamin C and E are famous examples. Furthermore, plant metabolites such as polyphenols have received considerable attention as natural antioxidants and are widely available in natural plants. Because the extracts of natural plants contain phenolic compounds, their antioxidant activity has been studied in various meats and meat products (Kumar *et al.*, 2015).

Perilla (*Perilla frutescens* L.) is an edible plant that is widely cultivated in Korea, Japan, and China (Asif, 2012; Gai *et al.*, 2017). Leaves of perilla are traditionally used as medicinal herbs due to its various bioactivities such as antioxidant, antimicrobial, anti-allergic, and anti-inflammatory activities (Jung *et al.*, 2017; Makino *et al.*, 2003; Ueda and Yamazaki, 2002). The color of the perilla leaves varies from dark green (green perilla) to reddish purple (red perilla), depending on its anthocyanin content (Asif, 2012). Red perilla leaves (*Perilla frutescens* var. *acuta*) contain a high concentration of phenolic compounds and its extract has the higher antioxidant activity than the extract of the leaves of green perilla (Asif, 2012). Given these results, this study aimed to evaluate the quality of enhanced pork loins manufactured by the injection of brine containing the extract of red perilla leaves.

Materials and Methods

Aqueous extraction of red perilla leaves

Dried leaves of red perilla were purchased in a local market. Dried leaves (50 g) of red perilla were mixed with distilled water (1.95 L) followed by the extraction in

a shaking water bath for 24 h at 70°C. The extract was centrifuged for 30 min at $6,710 \times g$ (CR 20B2, Hitachi Koki Co., Ltd. Japan), and the supernatants were recovered by gravity filtration using Whatman No. 4 filter paper (Whatman Inc., England). The filtrate was lyophilized (Ilshin Co., Korea). The resulting powdered extract of red perilla leaves (ERP) was stored in a deep freezer at -70°C until use.

Total phenolic content of ERP

The total phenolic content of ERP was measured using the Folin-Ciocalteu method described by Subramanian *et al.* (1965). A 0.1 mL sample in distilled water was added to 0.2 mL of Folin-Ciocalteu reagent and kept at 23°C for 1 min. A 5% sodium carbonate solution (3 mL) was added to the mixture and incubated in the dark at 23°C for 2 h. The absorbance of the mixture was measured at 765 nm using a spectrophotometer (DU 530, Beckman Instruments Inc., USA). The quantification of phenolics was based on a standard curve generated with the gallic acid and expressed as gallic acid equivalents (GAE).

Preparation of enhanced pork loins

Boneless pork loins (approximately 2.5-2.8 kg) were obtained from a local market (Korea). Pork loins were assigned to the injection of one of three treatments (control, brine containing no ERP; ERP 0.2, brine containing 2 g of ERP per kg of pork loin; and ERP 0.4, brine containing ERP at 4 g per kg of pork loin) (Table 1). The appropriate amount of ERP (2 or 4 g/kg of pork loin) was dissolved in brine before injection. Pork loins were weighed individually, and injected by the manual multiple-needle injector (a customized equipment) with brines (15% by weight). The injection process was repeated three times on the same day to manufacture three pork loins for each treatment. Pork loins were sealed in polyethylene bags and stored in a refrigerator at 4°C for 24 h. Each pork loin was then cut into three pieces, which were individually packaged in polyethylene bags, and stored in a refrigerator at 4°C for 0, 3, and 7 d. On each storage int-

Table 1. Formulation (%) of the injected ingredients and additives in the enhanced pork loins

	Control	ERP 0.2	ERP 0.4
Water	15	15	15
Sodium Chloride	0.2	0.2	0.2
Sodium pyrophosphate	0.3	0.3	0.3
ERP ¹⁾	-	0.2	0.4

¹⁾Extract of red perilla leaves

erval, a piece of each pork loin was cut into 2 cm slices, three of which were randomly selected for testing.

Color

The color (CIE L^* , a^* , and b^* values) of raw pork loin slices was measured using a colorimeter (CM-3500d, Minolta, Japan). Measurements were taken perpendicular to the surface of the slice with an illumination diameter of 30 mm at three different locations per sample. The results were analyzed using Spectra Magic Software (Minolta, Japan).

pH

The raw pork sample (1 g) was mixed with 9 mL of distilled water and homogenized (T25 basic, IKA GmbH & Co. KG, Germany) at $1,130 \times g$ for 1 min. The homogenate was centrifuged (Continent 512R, Hanil Co., Ltd., Korea) at $2,265 \times g$ for 10 min and the supernatants were recovered by gravity filtration using Whatman No. 4 filter paper (Whatman Inc., England). The pH of each filtrate was determined measured with a pH meter (SevenEasy, Mettler-Toledo, Switzerland) which was pre-calibrated using standard buffers (pH 4.01, 7.00, and 9.21).

Cooking loss

The pork loin slices were cooked in an electric steam oven (EON-C305CSM, Tongyang Magic Co., Korea) at 180°C for 25 min until the internal temperature of the pork loin reached 75°C , and subsequently cooled at room temperature for 30 min. Cooking loss was calculated by the weight loss (%) of each slice after cooking.

Lipid oxidation

Lipid oxidation of cooked slices of pork loin was monitored by the detection of malondialdehyde (MDA). This procedure was conducted according to the method described by Jung *et al.* (2016). For this analysis, MDA was extracted from the samples with acetonitrile as follows. The sample (3 g) was homogenized with 6 mL of distilled deionized water and 50 μL of 7.2% 2,6-di-*tert*-butyl-4-methylphenol in ethanol using a homogenizer (T25 basic) at 16,000 rpm for 1 min. Next, 500 μL of the homogenate was transferred into an Eppendorf tube, and 100 μL of 6 M NaOH solution (final concentration: 1 M) was added for the alkaline hydrolysis of the protein-bound MDA. The tubes were incubated in a water bath at 60°C for 45 min. After cooling in ice for 5 min, 1 mL of acetonitrile was added to the tube, and the mixture was vigorously

vortexed. The tube was centrifuged at $13,000 \times g$ for 10 min (HM-150IV, Hanil Co., Ltd., Korea). The upper clear phase of the supernatant contained the MDA extract. As an MDA standard, the solution of 1,1,3,3-tetraethoxypropane (3.2 mM) was diluted with distilled to a concentration of 0.1, 0.2, 0.4, 0.8, or 1.6 mM. Subsequently, 1 mL of the MDA extract, standard, or deionized water (blank) was passed through a 0.2- μm PVDF syringe filter (Whatman), and the filtrate was collected in a vial. The concentration of MDA was analyzed by HPLC (ACME 9000, Younglin Instruments Inc., Korea), using an Atlantis T3 C18 RP column (4.6×250 mm, 5 μm particles) with a mobile phase consisting of 30 mM K_2HPO_4 (pH adjusted to 6.2 with H_3PO_4). The isocratic flow rate of the mobile phase was 1.2 mL/min, and the injection volume was 50 μL . The column temperature was maintained at 35°C and the UV/Vis detector was set to a wavelength of 254 nm. The concentration of MDA in each sample was expressed in MDA mg/kg of pork loin.

Sensory evaluation of pork loins

Sensory evaluation of cooked pork loin was conducted independently during three sessions. Each session was arranged with pork loins from each batch. Pork loin slices were cooked at 180°C for 25 min using an electric steam oven (EON-C 305CSM). The cooked pork loin slices were reheated at 180°C for 5 min and cut into even slices immediately before sensory analysis. Pork loins were served on white glass plates to ten panelists who evaluated the color, flavor, taste, odor, tenderness, and overall acceptability of the pork loins. Every parameter but odor was ranked on a 9-point hedonic scale (1 = extreme dislike, 9 = extreme like).

Statistical analysis

This study was conducted in triplicate. Data was analyzed using the PROC GLM procedure in a randomized complete block design (batch as a block). The experimental unit was a slice of pork loin. The major effect included in the statistical model was ERP concentration. In the analysis of the data from the sensory evaluation, the panel was included in the model as a random effect. Specific comparisons were made by Tukey's multiple range test when the main effect was significant. Results were reported as least-square mean values and the standard error of the least-square means (SEM). Statistical significance was defined as $p < 0.05$. SAS software (version 9.3, SAS Institute Inc., USA) was used for statistical analyses.

Results and Discussion

Color of the enhanced pork loins

The L^* -value of raw pork loins was influenced by brine injection containing ERP (Table 2). ERP 0.2 had a significantly lower L^* -value than the control at 4 and 7 d of storage ($p < 0.05$). In addition, the L^* -value of ERP 0.4 was lower than that of the control for all storage days ($p < 0.05$). There were no significant differences between the L^* -values of ERP 0.2 and ERP 0.4 for all storage days ($p > 0.05$). During storage periods, no variance in L^* -values was found in the control group. The L^* -values of ERP 0.2 and ERP 0.4 at 4 d of storage were significantly decreased compared with those in 0 d of storage ($p < 0.05$). However, there was no significant differences of the L^* -values of ERP 0.2 and ERP 0.4 between 0 and 7 d of storage ($p > 0.05$). ERP 0.2 and ERP 0.4 had significantly higher a^* and b^* -values compared to that of the control for all storage days ($p < 0.05$). There were no changes in the a^* and b^* -values across all treatments during storage periods except for the b^* -value of the control ($p > 0.05$).

The leaves of red perilla contains anthocyanin, which is composed of red, purple, and blue colored pigments (Asif, 2012; Gai *et al.*, 2017; Jung and Joo, 2013). Lee *et al.* (2016) reported that the addition of brown soybean extracts containing a large quantity of anthocyanins into pork patties resulted in the decrease of L^* -values and the increase of a^* -values. Previous studies have also reported the decrease of L^* -values and the increase of a^* -values due to naturally sourced anthocyanins added to meat (Ganhao *et al.*, 2010; Kim *et al.*, 2015). Therefore, the addition of natural preservatives in meat and meat products leads

to change the color due to their pigmentation. The color of meat is an important quality parameter because consumers generally judge the freshness of meat by the meat color. Generally, pork with the highest L^* -values and the lowest a^* -values, which appear similar to pale, soft, and exudative (PSE) meat, is considered poor-quality pork although it is not PSE (Jung *et al.*, 2015). In the present study, ERP decreased the L^* -value and increased the a^* -value of the enhanced pork loin. Therefore, it may be used for the improvement of the color of pork loins.

pH and cooking loss of the enhanced pork loins

pH is an important quality parameter in meat. At pH values near that of the isoelectric point of muscle protein (approximately 5.2-5.4), the water holding capacity of the tissue diminishes due to the decrease in net charges (Huff-Lonergan and Lonergan, 2005). The injection of brine containing ERP did not influence the pH of raw pork loins at 0 and 4 d of storage ($p > 0.05$, Table 3). However, ERP 0.4 had significantly a lower pH than that of the control after 7 d of storage ($p < 0.05$). There was no change in the pH of the control and ERP 0.2 during the storage periods ($p > 0.05$). Although the pH of ERP 0.4 changed during storage, no significant difference in pH was found between 0 and 7 d of storage. The pH of the red perilla extract solution was approximately 5.6 (data not shown). Lee *et al.* (2015) reported that the pH of red perilla extract ranged from 5.4 to 5.6 that its subsequent addition to beef patties at a concentration of 6 g/kg decreased the pH of beef patty. ERP was added at lower concentrations than in previous works (Lee *et al.*, 2015). In the present study, sodium pyrophosphate was also injected into the pork

Table 2. Color (CIE L^* , a^* , and b^* values) of the enhanced pork loins over 7 days of storage

	Storage (d)	Control	ERP 0.2 ¹⁾	ERP 0.4 ¹⁾	SEM ²⁾
L^* value	0	50.57 ^a	45.52 ^{abx}	41.99 ^{bx}	1.924
	4	51.51 ^a	38.77 ^{by}	34.64 ^{by}	1.418
	7	51.97 ^a	44.46 ^{bx}	43.69 ^{bx}	1.222
	SEM ³⁾	0.689	1.605	1.942	
a^* value	0	6.16 ^b	9.05 ^a	8.29 ^a	0.458
	4	6.18 ^b	10.49 ^a	9.53 ^a	0.479
	7	7.62 ^b	9.90 ^a	9.72 ^a	0.459
	SEM ³⁾	0.470	0.458	0.529	
b^* value	0	14.93 ^{bxy}	18.43 ^a	17.26 ^a	0.403
	4	14.72 ^{cy}	20.22 ^a	17.94 ^b	0.530
	7	16.30 ^{cx}	19.88 ^a	18.23 ^b	0.458
	SEM ³⁾	0.390	0.536	0.472	

¹⁾See Table 1. ^{2,3)}Standard errors of the least square mean (n=9)

^{a-c)}Different letters within the same row differ significantly ($p < 0.05$).

^{x-y)}Different letters within the same column differ significantly ($p < 0.05$).

loins. As an alkaline additive, sodium pyrophosphate has the capacity to buffer pH (Lee *et al.*, 2018). Therefore, the influence of ERP on the pH of pork loins is minor with the exception of the low pH of ERP 0.4 after 7 days of storage. The cooking loss of pork loins did not vary by treatment or storage period (Table 4).

Lipid oxidation of the enhanced pork loins

The lipid oxidation of cooked pork loins was measured by detecting MDA, an abundant secondary product of lipid oxidation (Jung *et al.*, 2016). The rate of lipid oxidation in raw meat increases with storage time, and highly oxidized raw meat samples show a substantial degree of lipid oxidation after cooking (Ferioli *et al.*, 2008). The MDA contents of cooked pork loins did not vary by treatment before storage (Table 5). However, ERP 0.2 and ERP 0.4 contained less MDA than the control sample after 4 and 7 days of storage ($p < 0.05$). Furthermore, the MDA contents of ERP 0.2 and ERP 0.4 at 4 and 7 d of storage did not differ significantly compared to that of the sample before storage, while the MDA contents of the control increased over time. These results indicate that ERP inhibited the lipid oxidation process in the enhanced pork loins. Lee *et*

al. (2015) reported that the lipid oxidation of beef patties was inhibited when an aqueous extract of red perilla was used as an additive. Phenolic compounds within ERP are responsible for this antioxidant activity.

ERP contained phenolic compounds at a concentration of 71.76 mg GAE/g (data not shown). Red perilla leaves contain several phenolic and flavonoids compounds (Asif, 2012). Previous studies reported that ERP contains three phenolic acids, including coumaroyl tartaric acid, caffeic acid, and rosmarinic acid, and six flavones, including apigenin 7-O-caffeoylglucoside, scutellarein 7-O-diglucuronide, luteolin 7-O-diglucuronide, apigenin 7-O-diglucuronide, luteolin 7-O-glucuronide, and scutellarein 7-O-glucuronide (Asif, 2012). In addition, malonylshisonin is a key anthocyanin in the water extract of red perilla (Asif, 2012). Phenolic compounds can inhibit lipid oxidation by scavenging free radicals and therefore preventing the initiation of the process and by donating an electron to terminate the radical chain reaction (Rice-Evans *et al.*, 1997). Furthermore, unlike vitamin C and E, it can act as a chelator for transition metal ions (Nimse and Pal, 2015). Transition metal ions in meat, especially free iron ions, promote lipid oxidation through Fenton's reactions (Fe^{2+} or

Table 3. pH of the enhanced pork loins during 7 days of storage

Storage (d)	Control	ERP 0.2 ¹⁾	ERP 0.4 ¹⁾	SEM ²⁾
0	6.02	5.90	5.90 ^y	0.063
4	5.97	6.03	6.07 ^x	0.039
7	6.07 ^a	5.98 ^{ab}	5.92 ^{by}	0.034
SEM ³⁾	0.055	0.039	0.035	

¹⁾See Table 1. ^{2,3)}Standard errors of least square mean (n=9)

^{a,b)}Different letters within the same row differ significantly ($p < 0.05$).

^{x,y)}Different letters within the same column differ significantly ($p < 0.05$).

Table 4. Cooking loss (%) of the enhanced pork loins over 7 days of storage

Storage (d)	Control	ERP 0.2 ¹⁾	ERP 0.4 ¹⁾	SEM ²⁾
0	23.43	25.37	27.27	4.246
4	29.50	32.34	26.55	1.802
7	27.16	28.42	27.90	2.523
SEM ³⁾	2.269	2.895	3.756	

¹⁾See Table 1. ^{2,3)}Standard errors of least square mean (n=9)

Table 5. Malondialdehyde content (mg/kg) of the cooked enhanced pork loins over 7 days of storage

Storage (d)	Control	ERP 0.2 ¹⁾	ERP 0.4 ¹⁾	SEM ²⁾
0	0.46 ^y	0.57	0.57	0.033
4	0.80 ^{ax}	0.56 ^b	0.60 ^b	0.049
7	0.89 ^{ax}	0.64 ^b	0.55 ^b	0.052
SEM ³⁾	0.040	0.024	0.044	

¹⁾See Table 1. ^{2,3)}Standard errors of least square mean (n=9)

^{a,b)}Different letters within the same row differ significantly ($p < 0.05$).

^{x,y)}Different letters within the same column differ significantly ($p < 0.05$).

Table 6. Sensory properties of the cooked enhanced pork loins over 7 days of storage

	Storage (d)	Control	ERP 0.2 ¹⁾	ERP 0.4 ¹⁾	SEM ²⁾
Color	0	4.92	4.48	4.56	0.212
	4	5.37	4.56	4.70	0.277
	7	4.86	5.24	4.83	0.307
Taste	0	6.12 ^a	4.44 ^b	3.88 ^b	0.300
	4	5.81 ^a	4.52 ^b	4.41 ^b	0.284
	7	6.24 ^a	5.55 ^{ab}	4.48 ^b	0.322
Flavor	0	5.24 ^a	4.32 ^b	3.60 ^b	0.253
	4	5.48 ^a	4.30 ^b	4.07 ^b	0.285
	7	5.69	5.86	4.93	0.294
Tenderness	0	5.48	4.84	4.68	0.351
	4	5.15	5.44	5.78	0.317
	7	5.86	4.76	5.10	0.328
Overall acceptability	0	6.16 ^a	4.28 ^b	3.88 ^b	0.277
	4	5.92 ^a	4.52 ^b	4.19 ^b	0.287
	7	6.07 ^a	5.17 ^{ab}	4.52 ^b	0.317

¹⁾See Table 1. ²⁾Standard errors of least square mean (n=9)

^{a-c}Different letters within same row differ significantly ($p < 0.05$).

Fe³⁺/H₂O₂), in which free iron ions generate hydroxyl radicals by reacting with hydrogen peroxides, resulting in the generation of lipid and peroxy radicals from lipid hydroperoxides (Jung *et al.*, 2012; Nimse and Pal, 2015).

Sensory evaluation of the enhanced pork loins

The scores for both the color and tenderness of the cooked pork loins were not significantly different between each of the treatments at each of the storage intervals (Table 6). The taste scores of ERP 0.2 and ERP 0.4 were significantly lower than those of control at 0 and 4 d of storage ($p < 0.05$). However, the taste score of ERP 0.2 was not significantly different than that of the control after seven days of storage ($p > 0.05$). Similar results were attained for the flavor of the cooked pork loins. Although ERP 0.2 and ERP 0.4 had significantly lower scores for flavor compared with those of the control at 0 and 4 d of storage ($p < 0.05$), there was no difference between the flavor scores for all treatment after 7 d of storage. For overall acceptability, significantly lower scores were found in ERP 0.2 and ERP 0.4 compared to that of the control at 0 and 4 d of storage ($p < 0.05$), however, ERP 0.2 was no different than the control at 7 d of storage. These data show that enhancing pork loins with ERP at a concentration of 4 g/kg (ERP 0.4) consistently resulted in the decrease in their sensory desirability. Mo *et al.* (1999) reported that food treated in a similar fashion had a lower sensory score due to the unique flavor of red perilla leaves. However, ERP 0.2 showed similar sensory properties to

the control after seven days of storage despite its lower scores after 0 and 4 d of storage. These results might be attributed to the inhibitory action of red perilla extract toward lipid oxidation. In the present study, lower levels of lipid oxidation were found in ERP 0.2 compared than in the control.

Conclusion

Enhanced pork loins manufactured by the injection of a brine containing ERP had decreased L^* -values and increased a^* -values. ERP contains phenolic compounds at a concentration of 71.76 mg GAE/g, which helped to inhibit the lipid oxidation process in these samples. Although the sensory properties of the enhanced pork loin were adversely affected by ERP, ERP 0.2 was ranked similarly to the untreated control sample after storage for 7 d. Therefore, the present study revealed that enhanced pork loins with improved oxidative stability can be produced using ERP as a natural antioxidant.

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