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A Thesis for the Degree of Master of Science

Improvement of Oxidation Stability of Enhanced Pork Loin by Red Perilla Leaf Extract

자소엽 추추물을 이용하여 산화 안정성이 증진된 최소가공 돈육 등심 개발

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List of abbreviations

WHC	Water Holding Capacity
a_w	Water Activity
USDA	United States Department of Agriculture
FSIS	Food Safety and Inspection Service
MDA	Malondialdehyde
ERP	Extract of red perilla leaf (*Perilla frutescens* var. *acuta*)

I. General Introduction

Meat is very nutritious food because it contains various nutrients such as protein, fat, vitamin, and mineral (Biesalski, 2005). In addition, meat is tasty, and the intake of meat leads to pleasure and satisfaction of consumers (Piazza *et al.*, 2015). For these reasons, the consumption of meat is being increased, and consumers are demanding high quality meat (Lee *et al*., 2016a).

The factors that determine meat quality are color, flavor, tenderness, and juiciness (Aaslyng *et al*., 2003). Meat color influences the meat purchasing decision more than other factors because consumers usually use meat color as an indicator of freshness (Mancini and Hunt, 2005). The color of meat is determined by the amount of myoglobin, major meat pigment, and their redox state (Mancini and Hunt, 2005). The meat color is largely divided into three types; 1) the purple color derived from deoxymyoglobin (Mb), 2) the bright red color derived from oxymyoglobin (MbO_2), which is prefers to consumer because of being considered to fresh meat, and 3) the brown color derived from metmyoglobin (MetMb), which is undesirable by consumer due to being considered non-fresh products (Rosenvold and Andersen, 2003). Tenderness, juiciness and flavor, which are important factors that affects repurchase of meat because it affects sensorial quality of meat (Jayathilakan *et al.*, 2007; Lee *et al.*, 2016a). The tenderness of meat is the most important factor affecting the palatability of the consumer (Lee *et al.*, 2009). The tenderness of meat depends on the amount of intramuscular fat and connective tissue, and muscle fiber type

(Lee *et al*., 2009; Sajid Arshad *et al*., 2016). The juiciness refers to the amount of liquid that comes out when chewing meat (Moon, 2002). The juiciness of meat is influenced by water content in the meat related with their water holding capacity (WHC) and intramuscular fat (Offer and Trinick, 1983). Flavor is influenced by various flavor precursors such as free amino acids, nucleotides, reducing sugars, and fatty acids and the volatile compounds generated through Maillard reaction and Strecker degradation (Kook *et al.*, 2009). However, the meat quality is generally deteriorated during processing and storage. Therefore, the improvement or preservation of meat quality is very important.

The major detrimental factors for meat quality are oxidation and microbial growth during storage of meat (Oh *et al*., 2013). Lipid oxidation is a major cause of deterioration in the meat quality, and it causes unpleasant odors as well as changes in flavor, texture and color (Kang *et al*., 2006). In addition, Joo *et al*. (2001) reported that it is important to inhibit the growth of microorganisms in meat because it causes not only decrease of quality but also decomposition of meat. Therefore, lipid oxidation and microbiological tests were used for measuring meat quality during storage (Jin and Choi, 2001).

Several methods have been used to prevent deterioration of meat quality. One of them is packaging. Kang *et al*. (2006) used vacuum packaging for increasing shelf-life of duck meat. Modified atmosphere packaging (MAP), which removes the air in the package and replaces the gases such as O_2, N_2, and CO_2, also used. It makes slow the respiration rate of meat, inhibits degradation by enzymes, and reduces the growth of microorganisms (Chae *et al.*, 2011). Another one is aging which is to keep the meat in the temperature above the

freezing point for a long time and can improve the tenderness, taste, and flavor of meat (Lee *et al.*, 2015a). Generally, dry and wet aging are widely used (Smith *et al*., 2008). Recently, enhanced meat is also known to one of the method to improve meat quality. Enhanced meat is the meat injected the solution containing water with phosphate, salt, sodium lactate, and antioxidants into fresh meat (Hayes *et al.*, 2006). Ingredients in solutions can play various roles. Phosphate increases WHC (Moon *et al.*, 2002) and salt prolongs shelf life (Matthews and Strong, 2005). Sodium lactate improves the color stabilization of meat and meat product (Kim *et al*., 2006) and antioxidant also increases shelf life by preventing lipid oxidant.

However, there is not much literature on enhanced meat. We consider that enhanced meat as one of the potential ways to improve the quality of meat. Therefore, the objective of this study is to produce the enhanced pork loin with the improvement of oxidation stability by injecting natural antioxidant.

II. Literature Review

1. Enhanced meat

Enhanced meat means the fresh meat reserving the improved quality by a minimum process (Hayes *et al*., 2006). Generally, the quality enhancement of fresh meat is conducted with an injection of solution containing water, salt, phosphates, potassium or sodium lactate, antioxidants, and varying flavor enhancers (Davis *et al*., 2004; Sherman *et al*., 2009). By adding these ingredients, it is possible to prevent color and flavor changes as well as improve juiciness, tenderness, shelf life, product yields and flavor (Kim *et al*., 2006). The important purpose of enhancement is not for improving low meat quality, but improving the overall eating experience of fresh meat during retail display and decreasing variation of meat quality (Miller, 1998).

Each non-meat ingredients added into meat plays many roles. Water, which is basic ingredient for enhanced meat, plays a role of solvent, increases the yield of the meat, and makes up for water loss during cooking (Xiong, 2005). Salt can improve flavor, texture, and water holding capacity (Vangnai *et al*., 2014). Phosphates increase water retention and cooking yield by increasing pH of meat (Pietrasik and Janz, 2009). Moreover, Salt and Phosphate interact with each other and make synergistic effect (Detienne *et al.,* 2003). Lactate increase not only cooking yield and flavor but also antimicrobial effects (Jensen *et al.,* 2003). It is important that compounds such as salt, phosphate, and lactate have to be

completely blended in injection solution (Xiong, 2005). For this, the phosphate must be dissolved first because if phosphate is added later, it forms a clot (Heinz and Hautzinger, 2007).

The US Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) made regulation regarding enhanced meat (USDA, 2013). It noticed that total level of injection solution adding into meat and the information of ingredients in the solution must be notified on the meat label such as "containing up to (adding level of ingredient) % of a solution" followed by descending order of predominance ingredients in the final meat product (USDA, 2013).

These days, consumers prefer to lean and healthy products due to health problems and diseases such as obesity and hypertension (Jin and Yang, 2012). Although the production of leaner meat has been increased due to consumer`s requests, the leaner meat has negative effects on sensory aspect, especially flavor and juiciness compared with normal meat due to less fat (Sheard *et al*., 1999). As a result, enhanced meat technology has been used in poultry for several decades, but now it is becoming more common in other species such as beef and pork (Sheard *et al*., 1999; Knock *et al.*, 2006).

Table 1. Previous studies on enhanced meat.

Study	Meat	Additive	Effects
Robbins et al. (2003)	Beef	· 0.4% sodium chloride · 0.4% sodium tripolyphosphate	Increasing juiciness, tenderness, and overall acceptability
Kim et al. (2006)	Beef	· 2.5% lactate	Increasing color stability
Sheard and Tali (2004)	Pork	· 0.5% salt · 0.5% phosphate · 0.3% sodium bicarbonate	Increasing tenderness
Hayes et al. (2006)	Pork	· 0.5% salt · 0.3% β-lactoglobulin enriched fractions	Increasing tenderness, juiciness and taste

2. Role of ingredients

2.1 Phosphates

Phosphates, especially polyphosphates, are widely used in meat product because it has various beneficial effects such as increasing WHC, antioxidant activity, and antimicrobial effects (Moon, 2002; Houben and Tjeerdsma-van Bokhoven, 2004).

WHC is one of important factors for deciding meat quality (Huff-Lonergan and Lonergan, 2005). It relates to texture, tenderness, juiciness, and ultimately palatability of meat and meat products (Moon, 2002). In addition, it affects economical aspects related with the yield of meat and meat products (Offer and Trinick, 1983). There are many mechanisms that phosphates improve WHC. WHC of meat is affected by pH and the pH decline in meat results in the decrease of WHC with the net-charge reduction of muscle proteins. Phosphates which are generally used as ingredient for meat product is basic compound, because adding phosphates can increase the pH of meat (Alvarado and McKee, 2007). In addition, phosphates inhibit the binding of myosin and actin in myofibillar protein or depolymerization actomyosin complexes that increase the space for holding water molecules (Offer and Trinick, 1983; Wynveen *et al*., 2001).

Phosphates show antioxidant activity by preventing oxidation of lipid and protein (Mikkelsen *et al*., 1991). The antioxidant activity of phosphate is given in the chelation of metal ions that can catalyse lipid and protein oxidation

(Mikkelsen *et al*., 1991). Phosphatse have potential antimicrobial activity (Zaika *et al*., 1997). Previous studies reported that polyphosphate increased shelf life in fresh and cured meat by delay microbial growth (Molin *et al*., 1987). It may function as antimicrobial agent by binding heavy metal ions that is essential for microbial growth (Houben and Tjeerdsma-van Bokhoven, 2004)

The addition of an appropriate amount of phosphate has a positive effect on the meat, but excessive addition of it may cause imbalance of mineral such as magnesium and calcium in the body and increase the risk of bone related diseases (Kim and chin, 2010). Accordingly, USDA regulates that the phosphate of the final product should not exceed 0.5% (Miller, 1998).

2.2 Salt

Salt, especially sodium chloride, has been used for a long time in meat product industries. The roles of salt in meat and meat product are; 1) inhibiting microbial growth, 2) enhancing WHC, 3) increasing emulsion stability, and 4) improving flavor (Park and Kim, 2016).

The most important role of salt is to increase shelf life (Matthews and Strong, 2005). The addition of salt decreases a_w and increases osmotic pressure in which the growth of microorganisms is obstructed (Tarté, 2009). Several studies reported inhibition of microorganism growth through adding salt in meat and meat product (Andres *et al.*, 2005; Park and Kim, 2016). In addition, salt improves WHC of meat and meat product. When salt is added to the meat,

anions penetrated into myofibrils and consequently bind with the positively charged group of muscle proteins. It can increase the net charges among muscle proteins and thereby increase the space for water retention (Offer and Trinick, 1983; Hah *et al*., 2005).

The flavor perception of salt, in particular saltiness, is formed by Na^+ cations (Ruusunen *et al*., 2001). It serves not only as a flavor contributor, but also as a promoter of other flavor ingredients (Tarté, 2009). The intensity of flavor is influenced by the salt content; however, it is also influenced by the fat content because fat is jointly contributed with the salt in meat products (Ruusunen and Puolanne, 2005). The addition of salt increased the salty taste of products containing more fat (Matulis *et al*., 1995).

There is no restriction on the amount of salt in meat and meat products. However, it is important to add an appropriate amount of salt because an excessive amount of salt negatively effects on the sensory aspect and a risk of various diseases such as cardiovascular diseases (De Wardener and MacGregor, 2002).

2.3. Sodium lactate

Sodium lactate has many positive effects on meat quality such as increasing color stability, meat juiciness, and meat flavor and decreasing microbial growth (Miller, 1998). The addition of sodium lactate to meat results in color stabilization of meat and meat product (Kim *et al*., 2006). There are

several mechanisms to maintain meat color. First, Ramanathan *et al*. (2011) reported that sodium lactate replenishes NADH via lactate dehydrogenase activity, resulting in increasing metmyoglobin reduction. The other mechanism is that sodium lactate increases the pH, which prevents oxidation of myoglobin and prevents formation of metmyoglobin (Miller, 1998). Sodium lactate has been shown to improve meat juiciness related to improvement of WHC by increasing pH (Alvarado and McKe, 2007). Moreover, the shear-force of meat can be increase with the decrease of WHC (Kim and Kim, 2016).

Another purpose of sodium lactate addition is to improve flavor (Lin and Lin, 2002). When sodium lactate is added to the meat, it not only enhances the flavor but also reduce the development of off-flavors derived from lipid oxidation because it acts as radical scavenger (Miller, 1998). Also, Lin and Lin (2002) reported that the addition of sodium lactate prolonged the lag phase of aerobic microorganisms and consequently increase the storage stability and decreasing off-odor derived from spoilage.

Several studies reported that sodium lactate could suppress microbial growth (Papadopoulos *et al.*, 1991). Sodium lactate decreases a_w, which effects on the microbial growth (Maca *et al*., 1997; Kim and Jo, 2010). Also, lactic acid decomposed from sodium lactate is incorporated into the cell of microorganism, and then delays the normal metabolic process which produce energy in the cell (Miller, 1998). As a result, the shelf life can be extended by inhibiting microbial growth (Stekelenburg and Kant-Muermans, 2001).

Although lactate is added to food in many cases, it should be used with

caution because the allowable range is currently determined. FSIS permits the use of sodium and potassium lactate in meat and meat products, except infant food, for preventing the growth of certain pathogens. FSIS regulated the amount of sodium and potassium lactate in meat and meat product, which can be used up to 4.8% for inhibiting pathogen growth and up to 2% for flavor enhancement (USDA, 2015).

2.4. Antioxidant

Lipid oxidation of meat results in the decrease of sensorial and nutritional quality with the generation of rancid flavor and toxic compound (Peña-Ramos and Xiong, 2003). Lipid oxidation is catalyzed by many factors such as light, heat, metal ions, oxygen, and free radicals (Kim *et al*., 2012a).

The attack of free radicals begins lipid oxidation and continuously occurs with the radical chain reaction (Cho *et al.,* 2006). Lipid oxidation produces reactants such as pentanal, hexanal, 4-hydroxynonenal, and malondialdehyde (MDA) via a three-step mechanism, an autocatalyic mechanism of free radicals (Table 2) (Fernández *et al*., 1997). To prevent lipid oxidation in meat and meat products, commercial synthetic antioxidants or natural antioxidants have been used (Nassu *et al*., 2003). Various kinds of commercial synthetic antioxidants such as trihydroxybutyrophenone (THBP), nordihydroguaiaretic acid (NDGA), butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), and tert-butylhydroquinone (TBHQ) are used for preventing oxidation (Tang *et al.,* 2001). However, the use of synthetic antioxidants has been excluded in meat

industries because of consumer's concern about toxic substances derived from synthetic antioxidants. Therefore, the interest of natural antioxidants derived from natural plant has been increased (Park *et al*., 2012). Natural plants have many phytochemicals including phenolics, flavonoid, and terpenoids, which prevent oxidation (Kim *et al.,* 2012b). Natural antioxidants inhibit lipid oxidation by two mechanisms; 1) scavenging free radical, 2) chelation activity of transition metal ions (Rice-Evans *et al.,* 1997; Njus and Kelley, 1991).

There are many natural plants which can be used for antioxidant. Tomato has various non-nutritional antioxidants including flavonoids, carotenoids, and phenolics compounds as well as nutritional antioxidants including vitamin A, C, and E (Kim *et al*., 2013a). Banerjee *et al*. (2012) reported that broccoli contains antioxidant component such as ascorbic acid, flavonoids, carotenoid, vitamins and tocopherols. Previously, antioxidant potential of broccoli in the meat has been found by Kim *et al.* (2013b). Burdock contains phenolic compounds including actin, arctigenin, and cynarin which effect on free radical scavenging activity (Im and Lee., 2014). Accordingly, many researches on the antioxidant activity of burdock have been studied (Lee, 2016). Red perilla leaf (*Perilla frutescens* var. *acuta*) is one of the medicinal and edible plants in Asian countries. It is known to have high antioxidant capacity (Meng *et al*., 2008). In addition, it contains several effects such as anti-inflammation, anticancer activity, antimicrobial activity, and increasing phagocytosis (Sone *et al*., 2010). Therefore, it is frequently used as food ingredients (Meng *et al.,* 2008).

Table 2. Mechanism of lipid oxidation

I. Initiation:	II. Propagation:	III. Termination:
(1) RH + O_2+R· + ·OOH	(2) R· + O_2 → ROO·	(5) R· + R· → R-R
	(3) RH + ROO· → ROOH + R·	(6) R· + ROO· → ROOR
	(4) ROOH → RO· + ·OH	(7) ROO· + ROO· → ROOR + O_2

(Modified from Fernández *et al.*, 1997)

Table 3. Natural ingredients for antioxidants in meat and meat products

Natural ingredient	Concentration	Meat product type	
Tomato	0.25%, 0.5%, 0.75% and 1.0%	Pork patties	Kim *et al*. (2013a)
Broccoli	0.1% and 0.5%	Beef patties	Kim *et al*. (2013b)
	1%, 1.5%, and 2%	Goat meat nugget	Banerjee *et al*. (2012)
Burdock	0, 100, 200, and 300 ppm	Lard	Kim and Choe (2004).
Red perilla leaf	0.6%	Beef patties	Lee *et al*. (2015)

III. Quality Properties of Pork Loin Injected with Red Perilla Leaf (*Perilla frutescens* var. *acuta*)

1. Abstract

The aim of this study was to produce the enhanced meat with the improvement of oxidation stability by injecting natural antioxidant into the pork loin. Three experiments were performed as below.

Experiment I: The natural antioxidants derived from tomato (*Solanum lycopersicum*), broccoli (*Brassica oleracea* var. *italica*), burdock (*Arctium lappa*), and red perilla leaf (*Perilla frutescens* var. *acuta*) were prepared by hot water extraction followed by lyophilization. The antioxidant activity was evaluated by total phenolic content, DPPH radical scavenging activity, and reducing power. The total phenolic compound was the highest in the extract of red perilla leaf (71.76 ± 2.112 mg GAE /g). The extract of red perilla leaf (*Perilla frutescens* var. *acuta*) (ERP) showed the lowest the half maximal effective concentration (EC_{50}) values of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and reducing power among the extracts of natural plants. The ERP had the highest antioxidant activity.

Experiment II: The optimal injection concentration of ERP was evaluated depending on the antioxidant activity of ERP in pork patty. The pork patties

were divided into 6 treatment groups: the addition of ERP at 0, 2, 4, 6, 8, and 10 g/kg concentration and measured the content of malondialdehyde (MDA) in cooked pork patty. The MDA content in pork patty added with ERP was significantly lower MDA content than that of pork patty ($P<0.05$). However, there was no significant difference of MDA content in pork patties added with ERP at a concentration of 2, 4, 6, 8, and 10 g/kg. As a result, the 2 g/kg and 4 g/kg of ERP were set to appropriate concentrations.

Experiment III: The quality properties of pork loin injected with ERP at a concentration of 2 g/kg and 4 g/kg (ERP 2 and ERP 4) were investigated and compared with non-injected pork loin or injection brine containing no ERP (ERP 0). The pH and cooking loss were no significantly different among all treatments. The MDA content of ERP 2 and ERP 4 were significantly lower than that of control and ERP 0 after storage for 4 days in cooked loin ($P<0.05$). ERP 2 and ERP 4 had lower L^*-value and higher a^* and b^*-values than that of non-injected and ERP 0 in both raw and cooked loins. However, the ERP 4 of odor and off-taste score was the highest among treatments during all storage periods. For these reason, in overall acceptability, the ERP 0 and ERP 4 had the highest and lowest scores, respectively. Also, no significant differences of overall acceptability scores were found among non-injected, ERP 0, and ERP 2 after storage for 7 days. From the all results, the injection of ERP at a concentration of 2 g/kg improved oxidation stability of pork loin during storage without adverse effect. The current study concluded that the production of enhanced pork meat with increase oxidative stability can be possible by the injection of ERP as natural antioxidant.

2. Introduction

Quality is the main factor that influences consumers' choice of meat. However, meat quality deteriorate during storage. In order to maintain and enhance the meat quality before consumption, various methods such as packaging and processing can be used (Wilkinson *et al*., 2006; Campbell *et al*., 2001). Recently, the interest in the enhanced meat has been increased. Enhanced meat means the fresh meats which are increased its quality by minimum process such as injection of solution containing water, phosphate, salt, sodium lactate, and antioxidants (Davis *et al*., 2004; Hayes *et al*., 2006).

Lipid oxidation is one of major reasons for quality deterioration and shelf life decline of meat with the changes of flavor, color, texture, odor, and nutritional value (Georgantelis *et al*., 2007; Banerjee *et al*., 2012). Lipid oxidation is initiated by an attack of free radicals, and continuously proceeds with the radical chain reaction (Cho *et al.*, 2006). Especially, polyunsaturated fatty acids are more susceptible than saturated fatty acids because the carbon-hydrogen bond adjacent to double bond is weak and consequently, the hydrogen is easily abstracted to 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 in meat industries because of consumer’s concern about toxic substances derived from synthetic antioxidants

(Sayari *et al*., 2015). Natural antioxidants are mainly derived from natural plant. It is considered to be safer than synthetic antioxidants. However, the antioxidative activity of natural antioxidants is mostly weaker than that of synthetic antioxidants (Liang *et al.,* 2006). Therefore, the process for natural antioxidants possessing the high antioxidative activity have been searched (Kim *et al*., 2012a).

There are various antioxidants that can be derived from natural substances (Lee *et al*., 2012). Vitamin C and E are famous antioxidant. It inhibits lipid oxidation by scavenging free radicals, which obstructs the initiation of lipid oxidation (Rice-Evans *et al.,* 1997). And, Vitamin C and E can terminate radical chain reaction of lipid oxidation by stabilizing lipid radicals with the electron donation (Njus and Kelley, 1991). Polyphenols are also well known as antioxidant. Polyphenols can also scavenge free radicals and lipid radicals like vitamin C and E. In addition, polyphenols have chelation activity of transition metal ions while vitamin C and E do not have this activity (Sayari *et al*., 2015). Transition metal ions in meat, especially free iron ions, promote lipid oxidation through Fenton's reactions (Fe^{2+} or Fe^{3+}/H_2O_2), in which free iron ions generate hydroxyl radicals by reaction with hydrogen peroxides, and generation of lipid radical and peroxyl radicals from lipid hydroperoxides (Lee, 2010; Jung *et al*., 2012). For this reason, polyphenols received a lot of attention as natural antioxidants.

Polyphenols are widely distributed in plants. Various natural plants were studied as a candidate of natural antioxidants for meat and meat products. It was reported that tomatoes (*Solanum lycopersicum*) contain a large amount of

antioxidative compounds (Na and Joo, 2012). Among them, lycopene has the highest quenching rate constant with reactive oxygen species and alleviate the oxidative damages (García *et al.*, 2009). Tomato (*Solanum lycopersicum*) and its by-product showed antioxidant activity in various meat products such as sausage (Na and Joo, 2012), jerky (Kim *et al*., 2012c), pork patties (Kang *et al*., 2010), beef hamburger (García *et al*., 2009), and beef patty (Candogan, 2002).

Broccoli (*Brassica oleracea* var. *italica*) contains various antioxidants and nutrients such as vitamins, carotenoids, glutathione, rutin, quercetin, selenium, and flavonol glycosides (Zhang and Hamauzu, 2004; Park *et al*., 2014). Broccoli has high antioxidant activity based on the high phenolic content (Chu *et al.*, 2002). Banerjee *et al*. (2012) and Kumar *et al.* (2013) reported that goat meat nugget and emu meat nugget with addition of broccoli powder had enhanced antioxidant activity. However, unlike the studies in other foods such as Seolgiddeok (Cho, 2009), pound cake (Oh and Lee, 2011), and white bread (Lee, 2015b), the study of its antioxidant activity in meat and meat is insufficient.

Burdock (*Arctium lappa*) is a common edible and medicinal plant in Asia countries for centuries because it has numerous biological effects such as anticarcinogenicity, antimutagenicity, antiaging, and antioxidant capacity (Ferracane *et al*., 2010; Liu *et al*., 2012). In specific, it shows the highest antioxidant capacity with contents of phenolic compounds such as caffeic acid, tannin and chlorogenic acid (Chan *et al*., 2011). Tae *et al*. (2015 and 2016) reported that the addition of burdock powder to bread and castella resulted in

the decrease of lipid oxidation. In addition, glutinous rice *dasik* added with burdock (*Arctium lappa*) improved the antioxidant properties (Nam *et al*., 2016). However, there are few studies of the antioxidant effect of burdock in meat and meat products.

Red perilla leaf (*Perilla frutescens* var. *acuta*) has various function such as antimicrobial activity, anticancer activity, and excellent antioxidant effect (Hwang and Jang, 2001; Kim *et al*., 2007; Son *et al*., 2010). Red perilla leaf (*Perilla frutescens* var. *acuta*) has been used as a food additive and showed many functional properties in *Dongchimi* (Hwang and Jang, 2001), *Kakdugi* (Mo *et al*., 1999) and *vinaigrette* dressing (Ahn, 2011). However, *Perilla frutescens* var. *acuta* is also poorly studied as functional substance in meat and meat products.

The objective of this study is to produce the enhanced pork loin with the improvement of oxidation stability by injecting natural antioxidant. Therefore, three experiments were conducted. Firstly, the antioxidant activity of extracts of four natural substances including tomato, broccoli, burdock, and red perilla leaf (*Perilla frutescens* var. *acuta*) were investigated and one extract was chosen based on its antioxidant activity. Secondly, the natural substance, which was selected in experiment I, was added into the pork patties and investigated optimal injection concentration of the natural substance. Finally, the enhanced pork loin was manufactured by injection of natural substance as antioxidant and its quality properties were evaluated.

3. Material and Method

3.1. Antioxidant activity of extracts from natural plants

3.1.1. Preparation of each plant extracts

Tomato powder (baekjangsaeng, Seoul, Korea), broccoli powder (baekjangsaeng, Seoul, Korea), and burdock powder (baekjangsaeng, Seoul, Korea) were used as the candidate for natural antioxidants. Each powder (200 g) was added to 1.8 L of distilled water. 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). Mitures were extracted in a shaking water bath for 24 h at 70°C, then extract was centrifuged for 30 min at 6,710×g (CR 20B2, Hitachi Koki Co., Ltd. Japan). The supernatants were filtered using filter paper (Whatman No. 4, Whatman Inc., Maidstone, England). Each filtrate was lyophilized (Ilshin Co., Gyeonggi-do, Korea), and stored at −70°C until use.

3.1.2. Total phenolic content

Total phenolic content was measured by using the Folin-Ciocalteu method described by Subramanian et al. (1965). A 0.1 mL sample with distilled water was added to 0.2 mL Folin-Ciocalteu reagent and kept at 23°C for 1 min. Then, a 3 mL sodium carbonate (5%) was added to the mixture,

vortexed, 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., Fullerton, CA, USA). The quantification of natural phenolics was based on the standard curve using gallic acid, and expressed as gallic acid equivalents (GAE).

3.1.3. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity of each extracts was estimated by following the method of Blois (1958). Each extracts (1 mL) with distilled water was mixed with 1 mL of 0.2mM DPPH solution (in methanol) and then the mixture was left at room temperature for 30 min. Distilled water (1 mL) with 1 mL of 0.2 mM DPPH solution (in methanol) was used as the control. The absorbance of the mixture was measured at 517 nm using a spectrophotometer (DU 530, Beckman Instruments Inc., Fullerton, CA, USA). The percentage of DPPH radical scavenging was calculated from the following equation: Radical scavenging activity = [(absorbance of control - absorbance of sample) / absorbance of control] × 100. The half maximal effective concentration (EC_{50}) of each extracts for DPPH radical scavenging was calculated by interpolation from the data.

3.1.4. Reducing power

Reducing power of each extracts estimated according to the method Oyaizu (1986). The 0.2 mL of each extracts was mixed with 500 μL of 0.05 M

phosphate buffer (pH adjusted to 6.6) and then, 1% potassium ferricyanide (500 μL) was added. The mixture was heated in a water bath at 50°C for 20 min and cooled. After cooling, 10% trichloroacetic acid (500 μ L) was added and centrifuged at 10,000 ×*g* for 10 min (HM-150IV, Hanil Co., Ltd., Incheon, Korea). The supernatant (500 μL) was mixed with distilled water (500 μL) and 0.1% Ferric chloride (100 μL) and then the mixture was left in dark for 5 min. The absorbance of the mixture was measured at 700 nm using a spectrophotometer (DU 530, Beckman Instruments Inc., Fullerton, CA, USA). The half maximal effective concentration (EC_{50}) of each extracts for reducing power was calculated by interpolation from the data.

3.1.5. Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) and the significant differences between the mean values were identified with Tukey's multiple range test using SAS software at a confidence level of $P<0.05$ (SAS 9.3, SAS Institute Inc., Cary, NC, USA).

3.2. Evaluation of optimal injection concentration

3.2.1. Preparation of the pork patties

Ground pork loin was purchased in a local market (Daejeon, Korea) and mixed with water and additives (Table 4). Meat batter was divided into 6 treatment groups: The extract of red perilla leaf (*Perilla frutescens* var. *acuta*) (ERP) at 0, 2, 4, 6, 8, and 10 g/kg concentration. The meat batters for the three batches were prepared the same day and stored in a refrigerator at 4°C for 12 h prior to manufacture of patty. The meat batter (30 g) put into petri dish that is thickness of 1.0 cm and diameter of 5.0 cm and individually vacuum-packaged in a low-density polyethylene/nylon vacuum bags (10 cm × 10 cm; oxygen permeability of 22.5 mL/m^2/24 h atm at 60% RH/25°C; water vapor permeability of 4.7 g/m^2/24 h at 100% RH/25°C) using a vacuum-packaging machine (FJ-600XL; Hankook Fujee Industries Co., Hwaseong, Korea) at -650 mmHg. Three patties of each treatment/batch were prepared and cooked in a water-bath for 30 min at 100°C until internal temperature of the patties reached to 75°C.

Table 4. Formulation (%) of the pork patties

Ingredients	Treatment (g/kg)					
	0	2	4	6	8	10
Pork loin	90	90	90	90	90	90
Water	10	10	10	10	10	10
Total	100	100	100	100	100	100
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Phosphate	0.3	0.3	0.3	0.3	0.3	0.3
ERP[1]	-	0.2	0.4	0.6	0.8	1

[1)]ERP, Extract of red perilla leaf (*Perilla frutescens* var. *acuta*).

3.2.2. Lipid Oxidation

Lipid oxidation of meat samples was measured according to the method of Jung *et al*. (2016). A meat sample (3 g) was added with distilled water (6 mL) and 7.2% 2, 6-di-tert-butyl-4-methylphenol (in ethanol). The mixture was homogenized (T25 basic, IKA GmbH & Co. KG, Staufen, Germany) at 16,000 rpm for 1 min. Then, the homogenate (500 μL) and 6 M NaOH solution (200 μL) were transferred into micro-tube. The tubes were heated in a water bath at 60°C for 45 min, and then cooled at room temperature. After cooling, 1 mL of acetonitrile (ACN) was added and centrifuged at 13,000 ×*g* for 10 min (HM-150IV, Hanil Co., Ltd., Incheon, Korea). After that, the supernatant was collected into vial through 0.2 μm polyvinylidene difluoride (PVDF) syringe filter (Whatman PLC., Maidstone, UK). Ultimate 3000 HPLC system anaylzed the malondialdehyde (MDA) (Thermo Fisher Scientific Inc., Waltham, MA, USA) by using Atlatis T3 C18 RP column (4.6 × 250 mm, 5 μm particles) and

30 mM potassium phosphate dibasic (mobile phase, pH adjusted to 6.2 with phosphoric acid). The 50 µL of sample was injected with mobile phase following the flow rate of the 1.2 mL/min. The column temperature was kept at 35°C and UV/VIS detector was used to 254 nm. 1, 1, 3, 3-tetraethoxypropane solution in 0.1 M hydrochloric acid was used for standard of malondialdehyde (MDA) and expressed in mg MDA/kg of pork patty.

3.2.3. Statistical analysis

The whole experiment was triplicated. Data was analyzed using the PROC GLM procedure. The experimental unit was a patty. The statistical model included the main effect of the ERP concentration. Specific comparisons were performed by Tukey's multiple range test when the main effect was significant. Results are reported as least-square mean values and standard error of the least-square means (SEM). Statistical significance was considered for $P<0.05$. SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

3.3. The quality properties of enhanced pork loin manufactured by injection of brine containing ERP

3.3.1 Preparation of meat sample

Boneless pork loins (around 2.5-2.8 kg) were obtained from a local market (Daejeon, Korea). The ERP was dissolved in injection brine to be injected at a level equivalent 2 or 4 g/kg (Table 5). Pork loins were weighed individually and injected at a level equivalent to 15% of pork loins' weight by multiple-needle injector. The injection process was conducted with three times (three batches) in the same day. After injection, pork loins were sealed in polyethylene bags and stored in a refrigerator at 4°C for 24 h. Pork loins cut into three pieces. Pieces of pork loin were individually packaged by polyethylene bags and stored in a refrigerator at 4°C for 0, 3, and 7 days. Three pieces of each pork loin were randomly collected as replication samples in each storage day. The samples were cooked in electric steam oven (EON-C305CSM, Tongyang Magic Co., Korea) at 180°C for 25 min until internal temperature of the pork loin reached to 75°C.

Table 5. Formulation[1] (%) of the injected solution

Ingredients	Non-injected	ERP[2] (g/kg)		
		0	2	4
Water	-	15	15	15
Salt	-	0.2	0.2	0.2
Phosphate	-	0.3	0.3	0.3
ERP[2]	-	-	0.2	0.4

[1)]Weight/weight basis of non-injected meat.

[2)]ERP, Extract of red perilla leaf (*Perilla fruescens* var. *acuta*).

3.3.2 pH

The meat sample (1 g) was mixed with 9 mL of distilled water and homogenized (T25 basic, IKA GmbH & Co. KG, Staufen, Germany) at 1,130 $\times g$ for 1 min. The mixtures were centrifuged (Continent 512R, Hanil Co., Ltd., Incheon, Korea) at 2,265 $\times g$ for 10 min and filtered using filter paper (Whatman No. 4, Whatman PLC., Maidstone, UK). The pH value of each filtrate was determined using a pH meter (SevenEasy, Mettler-Toledo, Schwerzenbach, Switzerland) which was pre-calibrated using standard buffers (pH 4.01, 7.00, and 9.21).

3.3.3 Cooking loss

Cooking loss of pork loin was examined by the percentage weight loss of each meat sample after cooking (Lee *et al*., 2015c). Cooking loss was calculated as expressed below:

$$\text{Cooking loss (\%)} = \frac{\text{Weight before cooking} - \text{Weight after cooking}}{\text{Weight before cooking}} \times 100$$

3.3.4. Lipid oxidation

Lipid oxidation of meat samples was measured according to the method of Jung *et al*. (2016). A meat sample (3 g) was added with distilled water (6 mL) and 7.2% 2, 6-Di-tert-butyl-4-methylphenol (in ethanol). The mixture was homogenized (T25 basic, IKA GmbH & Co. KG, Staufen, Germany) at 16,000 rpm for 1 min. Then, the homogenate (500 μL) and 6 M NaOH solution (200 μL) were transferred into micro-tube. The tubes were heated in a water bath at 60°C for 45 min, and then were cooled at room temperature. After cooling, 1 mL of ACN was added and centrifuged at 13,000 ×*g* for 10 min (HM-150IV, Hanil Co., Ltd., Incheon, Korea). After that, the supernatant was collected into vial through 0.2 μm PVDF syringe filter (Whatman PLC., Maidstone, UK). Ultimate 3000 HPLC system analyzed the MDA (Thermo Fisher Scientific Inc., Waltham, MA, USA) by using Atlatis T3 C18 RP column (4.6 × 250 mm, 5 μm particles) and 30 mM potassium phosphate dibasic (mobile phase, pH adjusted to 6.2 with phosphoric acid). The 50 μL of sample was injected with mobile phase following the flow rate of the 1.2 mL/min. The column temperature was kept at 35°C and UV/VIS detector was used to 254 nm. 1, 1, 3, 3-tetraethoxypropane solution in 0.1 M hydrochloric acid was used for standard of MDA and expressed in mg MDA/kg of pork patty.

3.3.5. Surface Color

The surface color (CIE L^*, a^*, and b^* values which were represented lightness, redness, and yellowness, respectively) of pork loin was obtained using a colorimeter (CM-3500d, Minolta, Osaka, Japan). Each color values was measured at 3 random points per sample for minimizing the errors and the average was used as one replicate. The color value was automatically analyzed (Spectra Magic Software, Minolta, Osaka, Japan).

3.3.6. Sensory evaluation

Sensory evaluation of pork loin was conducted independently with three sessions as a replication. Each session was arranged with pork loin in each batch. Pork loin was reheated at 180°C for 5 min using electric steam oven (EON-C 305CSM, Tongyang Magic Co., Korea). Panelists of sensory evaluation were ten who have experience in sensory test of meat and meat product. Panelists evaluated the cooked samples for color, flavor, taste, odor, off-taste, tenderness, and overall acceptability. The color, flavor, taste, tenderness, and overall acceptability were measured by a 9-point hedonic scale (1 = extremely dislike, 9 = extremely like). The odor and off-taste were scored by a 9-point hedonic scale (1 = have no odor and off-taste, 9 = have strong odor and off-taste).

3.3.7. Statistical analysis

The study was conducted by triplicate with three batches. Data was analyzed using the PROC GLM procedure in a randomized complete block

design (batch as a block). The experimental unit was a piece of pork loin. The statistical model included the main effect of the ERP concentration. For analyzing data from the sensory evaluation, the panel was included in model as the random effect. Specific comparisons were performed by Tukey's multiple range test when the main effect was significant. Results were reported as least-square mean values and standard error of the least-square means (SEM). Statistical significance was considered for $P<0.05$. SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

4. Result and Discussion

4.1. Antioxidant activity of extracts from four natural plants

4.1.1. Total phenolic content of extracts

The contents of phenolic compounds in natural plant extracts are present in Table 6. The ERP had the highest phenolic compound at a concentration of 71.76 mg GAE/g compared to other extracts. No significant differences of total phenolic content were found among extracts from tomato (*Solanum lycopersicum*), broccoli (*Brassica oleracea* var. *italica*), and burdock (*Arctium lappa*). It has been reported that the red perilla leaf contained various phenolic compounds such as anthocyanin, rosmarinic acid, caffeic acid, and ferulic acid (Ha *et al*., 2012; Jun *et al*., 2014).

4.1.2. DPPH radical scavenging activity and reducing power of extracts

Most phenolic substances can remove free radicals by donation of electron (Jang *et al*., 2012). Therefore, in this study, antioxidant activity was evaluated by measuring both DPPH radical scavenging activity and reducing power.

DPPH radical scavenging activity was measured using the principle that DPPH radical, a kind of free radicals and known to cause oxidation, was removed by antioxidants (Jang *et al*., 2012). The half-maximal effective concentration (EC_{50}) of all extracts for scavenging DPPH radicals was shown

in Table 7. The EC_{50} values for scavenging of DPPH radicals of extracts from tomato, broccoli, burdock, and red perilla leaf were 9.61, 4.97, 3.78 and 0.93 mg/mL, respectively. The ERP had 4 to 10 times lower EC_{50} value than other extracts.

Reducing power is the reduction activity of oxidized molecules by donation of electron. The EC_{50} values for reducing power of extracts from tomato, broccoli, burdock, and red perilla leaf were 3.97, 4.08, 3.95, and 0.58 mg/mL, respectively (Table 7). There were no significant differences among extracts from tomato, broccoli, and burdock ($P>0.05$).

The ERP showed the highest antioxidant activity with the highest content of phenolic compounds compared with extracts of tomato, broccoli, and burdock. Previous studies reported the positive correlations in antioxidant activity, phenolic content, DPPH radical scavenging activity, and reducing power (Park *et al.*, 2011; Kim *et al.*, 2013c; Byun *et al.*, 2016). Based on result, the ERP was selected as a natural antioxidant for following experiment.

Table 6. Total phenolic content (mg GAE[1]/g) of natural plant extracts

Plant extracts	Total phenolic content
Tomato (*Solanum lycopersicum*)	8.95[b]
Broccoli (*Brassica oleracea* var. *italica*)	15.78[b]
Burdock (*Arctium lappa*)	15.32[b]
Red perilla leaf (*Perilla frutescens* var. *acuta*)	71.76[a]
SEM[2]	2.112

[1)]Gallic acid equivalent.

[2)]Standard error of mean (n=12).

[a,b]Different letters within the same column differ significantly ($P<0.05$).

Table 7. EC_{50} value for scavenging of DPPH[1] and EC_{50} value for Reducing Power[2] of natural plant extracts

Plant extracts	EC_{50} value of scavenging	
	DPPH (mg/mL)	Reducing Power (mg/mL)
Tomato (*Solanum lycopersicum*)	9.61[a]	3.97[a]
Broccoli (*Brassica oleracea* var. *italica*)	4.97[b]	4.08[a]
Burdock (*Arctium lappa*)	3.78[c]	3.95[a]
Red perilla leaf (*Perilla frutescens* var. *acuta*)	0.93[d]	0.58[b]
SEM[3]	0.049	0.043

[1)]The half maximal effective concentration of extracts for DPPH radical scavenging.

[2)]The concentration of extracts for obtaining 0.5 of absorbance by reducing power.

[3)]Standard error of mean (n=12).

[a-d]Different letters within the same column differ significantly ($P<0.05$).

4.2. Evaluation of optimal injection concentration

4.2.1. Lipid oxidation

The optimal injection concentration of ERP was evaluated based on the inhibition effect of lipid oxidation in pork patty. The ERP was added into pork patty at a concentration of 2, 4, 6, 8, and 10 g/kg. The MDA content in pork patties added with various concentrations of ERP was significantly lower than that of pork patty without ERP (Table 8, $P<0.05$). However, the increase of ERP concentration in pork patty did not suppress lipid oxidation further compared to ERP 2 g/kg (ERP 2) ($P<0.05$).

There are possible reasons for no significant differences of MDA content in pork patties even if the added concentration of ERP was increased. Previous studies have shown that the pork patties and pork sausages added with plant extracts at various levels had no difference in the MDA content at 0 day of storage (Lee *et al.,* 2004). Cha and Lee (2013) reported that the addition of a natural antioxidant, purple Kohlrabi (*Brassica oleracea* var. gongylodes), into beef showed inhibition effect of lipid oxidation after 10 day of storage. In addition, pork loin is known to have lower fat content than other muscles (Seong *et al.,* 2010). Song *et al.* (2014) reported that lipid oxidation in meat products is dependent on fat content and fatty acid composition. Therefore, it is considered that no significant differences of MDA content among the pork patties added with ERP at a concentration of 2, 4, 6, 8, and 10 g/kg due to no storage time and the low fat content of the pork loin used in this experiment. Based on the results, the concentration of 2 and 4 g/kg was decided as the

optimal injection concentration for manufacture of enhanced pork loin.

Table 8. Lipid oxidation (mg MDA[1]/kg) of pork patties added different concentration of extract of red perilla leaf (*Perilla frutescens* var. *acuta*)

Concentration of ERP[2] (g/kg)	Lipid oxidation
0	0.33[a]
2	0.25[b]
4	0.21[b]
6	0.22[b]
8	0.23[b]
10	0.26[b]
SEM[3]	0.013

[1)]MDA, Malondialdehyde

[2)]ERP, Extract of red perilla leaf (*Perilla frutescens* var. *acuta*).

[3)]Standard error of mean (n=18).

[a,b]Different letters within the same column differ significantly ($P<0.05$).

4.3. The quality properties of enhanced pork loin manufactured by injection of brine containing ERP into pork loin

The ERP was dissolved in injection brine to be injected at a level equivalent 2 or 4 g/kg of ERP into pork loin, and the quality properties of pork loin were evaluated during storage for 7 day at 4°C.

4.3.1. pH and cooking loss

The pH and cooking loss were measured during refrigerated storage at 4°C for 0, 4, and 7 day. No significant differences of pH were found among non-injected pork loin, injection brine containing no ERP (ERP 0), ERP 2, ERP 4 in all storage days (Table 9). The pH of non-injected pork loin was significantly decreased after storage for 4 and 7 days compared with that of 0 day ($P<0.05$). However, there was no change of pH in ERP 0 and ERP 2 during storage ($P>0.05$). Although the pH of ERP 4 was changed during storage, no significant difference was found between 0 day and 7 day.

The pH of meat effects on water-holding capacity of meat, and cooking loss has negative correlation with water-holding capacity (Huff-Lonergan and Lonergan, 2005; Lee *et al*., 2015b). In the present study, the pH was not different among treatments and consequently, the cooking loss of pork loin in all treatments was similar with no significant differences in all storage days (Table 10, $P>0.05$). During storage, the pH of non-injected and ERP 4 was

changed. However, the cooking loss of non-injected and ERP 4 was not different during storage (P>0.05).

Table 9. pH of pork loin added different concentration of extract of red perilla leaf (*Perilla frutescens* var. *acuta*)

Storage days	Non-injected	ERP[1] (g/kg)			SEM[2]
		0	2	4	
0	6.01[x]	6.02	5.90	5.90[y]	0.059
4	5.38[y]	5.97	6.03	6.07[x]	0.041
7	5.79[y]	6.07	5.98	5.92[y]	0.037
SEM[3]	0.026	0.055	0.039	0.035	

[1)]ERP, Extract of red perilla leaf (*Perilla frutescens* var. *acuta*).

[2)]Standard error of mean (n=12), [3)](n=9)

[x,y]Different letters within the same column differ significantly (P<0.05).

Table 10. Cooking loss (%) of pork loin added different concentration of extract of red perilla leaf (*Perilla frutescens* var. *acuta*)

Storage days	Non-injected	ERP[1] (g/kg)			SEM[2]
		0	2	4	
0	27.89	23.43	25.37	27.27	2.553
4	33.37	29.50	32.34	26.55	1.926
7	29.39	27.16	28.42	27.89	2.707
SEM[3]	3.399	1.927	3.146	3.812	

[1)]ERP, Extract of red perilla leaf (*Perilla frutescens* var. *acuta*).

[2)]Standard error of mean (n=12), [3)](n=9)

4.3.2. Lipid oxidation

Lipid oxidation in cooked pork loin was measured by MDA content that is an abundant secondary product of lipid oxidation (Jung *et al*., 2016). The MDA contents of ERP 2 and ERP 4 were not significantly different compared with that of non-injected and ERP 0 in 0 day of storage (Table 11). However, the MDA contents of ERP 2 and ERP 4 were significantly lower than that of non-injected and ERP 0 after storage for 4 and 7 days ($P<0.05$). In addition, the MDA contents of non-injected and ERP 0 were significantly increased with the increase of storage days ($P<0.05$). However, the no significant differences of MDA content between ERP 2 and ERP 4 were found in all storage days ($P>0.05$).

These results meant that ERP inhibited lipid oxidation of pork loin. The antioxidant activity of ERP in pork loin is derived from phenolic compounds. Red perilla leaf contains many phenolic compounds and flavonoids compounds (Lee et al., 2013). Previous studies which were study on natural antioxidants such as purple *Kohlrabi* (*Brassica oleracea* var. *gongylodes*) and dandelion extract found that phenolic compounds and flavonoid compounds have antioxidant activity and thus inhibit lipid oxidation (Cha and Lee, 2013; Choi et al., 2015).

These phenolic compounds are known as free radical scavengers that provide electrons and hydrogen ions to inhibit oxidation (Lee, 2010). Kim *et al*. (2013c) have also shown that phenolic compounds and antioxidant activities have positive correlations. Several studies have found the inhibition of lipid oxidation in meat by adding natural products containing a large amount of

phenolic compounds (An *et al*., 2008; Ha *et al*., 2012). Moreover, Lee *et al*. (2015b) reported that the addition of red perilla extract into beef patties effectively reduces lipid oxidation in beef. Base on this result, it can be concluded that the addition of ERP to meat inhibit lipid oxidation.

Table 11. Lipid oxidation (mg MDA/kg) of cooked pork loins added different concentration of extract of red perilla leaf (*Perilla frutescens* var. *acuta*)

Storage days	Non-injected	ERP[1] (g/kg)			SEM[2]
		0	2	4	
0	0.64^{ay}	0.46^{by}	0.57^{ab}	0.57^{ab}	0.033
4	0.87^{ax}	0.80^{ax}	0.56^{b}	0.60^{b}	0.048
7	0.84^{ax}	0.89^{ax}	0.64^{b}	0.55^{b}	0.050
SEM[3]	0.044	0.040	0.024	0.045	

[1)]ERP, Extract of red perilla leaf (*Perilla frutescens* var. *acuta*).

[2)]Standard error of mean (n=12), [3)](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).

4.3.3. Surface color

The surface color of raw and cooked pork loins was shown in Table 12. In the raw pork loin, the L^*-value of pork loin was influenced by brine injection containing ERP. ERP 2 and ERP 4 had lower L^*-value than non-injected and ERP 0 in all storage days (P<0.05). In contrast, the a^* and b^*-values were significantly higher in ERP 2 and ERP 4 than non-injected and ERP 0 after storage for 4 day (P<0.05). The L^*-values of non-injected and ERP 0 were not changed during storage periods. The L^*-values of ERP 2 and ERP 4 were significantly decreased after storage for 4 day. However, there was no

significant differences of the L^*-values of ERP 2 and ERP 4 at 0 day of storage compared to that of 7 days. No differences of a^*-values in all treatments were found during storage periods. The b^*-value of non-injected meat was significantly increased after storage for 7 day. However, no consistent change of the b^*-values in pork loins with brine injection (ERP 0, 2, and 4) was found during storage periods.

No significant difference of L^*-values in cooked pork loin from all treatments was found in 0 day of storage. However, ERP 2 and ERP 4 had low L^*-values compared to non-injected and ERP 0 after storage for 4 and 7 days ($P<0.05$). The a^*-value of ERP 4 was significantly higher than that of non-injected and ERP 0 while ERP 2 showed no significant difference of a^*-values with non-injected in 0 day of storage. ERP 2 and ERP 4 had significantly high a^*-values compared non-injected and ERP 0 after storage for 4 and 7 days. The b^*-values were not significantly different among non-injected, ERP 0, and ERP 2 whereas that was the highest in ERP 4 at 0 day of storage. After storage for 4 day, the b^*-values of ERP 2 and ERP 4 were significantly higher than that of non-injected and ERP 0. However, there was no significant difference of the b^*-values among all treatments in 7 day of storage. Except for the b^*-values of ERP 4, no changes of the L^*, a^*, and b^*-values in cooked pork loin were found in all treatments during the storage time.

These color changes of pork loin caused by brine injection containing ERP occur due to the anthocyanins and flavonoids contained in red perilla leaf (Lee *et al*., 2015b). The anthocyanins are red, purple, and blue colored pigments

(Hwang and Ki, 2013; Jung and Joo, 2013). Lee *et al*. (2016b) reported that the addition of brown soybean extract containing a large amount of anthocyanins into pork patties resulted in the decrease of L^*-value and the increase of a^*-value in pork patties. Previous studies have also found that an decrease of L^*-value and increase of a^*-value due to anthocyanins in added natural product as additives into meat (Ganhão *et al*., 2010; Kim *et al*., 2015). Chen *et al*. (1999) and Lee and Ahn (2005) reported that the addition of colored natural product into meat inevitably leads to change the color due to original color of natural product.

Table 12. Surface of raw and cooked pork loins added different concentration of extract of red perilla leaf (*Perilla frutescens* var. *acuta*)

	Storage days	Non-injected	ERP[1] (g/kg)			SEM[2]
			0	2	4	
			Raw meat			
L^*-value	0	50.31^{a}	50.57^{a}	45.52^{bx}	41.99^{bx}	1.834
	4	52.34^{a}	51.51^{a}	38.77^{by}	34.64^{by}	1.338
	7	50.08^{a}	51.97^{a}	44.46^{bx}	43.69^{bx}	1.186
	SEM[3]	1.129	0.689	1.605	1.942	
a^*-value	0	6.88^{bc}	6.16^{c}	9.05^{a}	8.29^{ab}	0.427
	4	7.34^{b}	6.18^{b}	10.49^{a}	9.53^{a}	0.448
	7	7.87^{b}	7.62^{b}	9.90^{a}	9.72^{a}	0.412
	SEM[3]	0.307	0.47	0.458	0.529	
b^*-value	0	14.86^{by}	14.93^{bxy}	18.43^{a}	17.26^{a}	0.386
	4	15.05^{by}	14.72^{by}	20.22^{a}	17.94^{a}	0.473
	7	15.33^{bx}	16.30^{bx}	19.88^{a}	18.23^{a}	0.433
	SEM[3]	0.258	0.39	0.536	0.472	
			Cooked meat			
L^*-value	0	71.49	74.65	71.01	66.29	1.535
	4	71.86^{a}	72.95^{a}	66.78^{b}	62.00^{c}	1.079
	7	73.48^{a}	74.23^{a}	67.69^{b}	66.53^{b}	1.236
	SEM[3]	1.154	0.707	1.394	1.841	
a^*-value	0	2.57^{bc}	1.24^{c}	2.98^{ab}	4.33^{a}	0.387
	4	1.97^{c}	1.86^{c}	3.83^{b}	6.05^{a}	0.290
	7	2.42^{b}	1.50^{b}	4.77^{a}	5.4^{1a}	0.348
	SEM[3]	1.154	0.707	1.394	1.841	
b^*-value	0	13.15^{b}	13.72^{b}	15.20^{ab}	16.86^{ax}	0.771
	4	13.65^{b}	13.28^{b}	16.41^{a}	17.45^{ax}	0.574
	7	14.10	13.54	14.09	13.19^{y}	0.558
	SEM[3]	0.596	0.519	0.706	0.634	

[1)]ERP, Extract of red perilla leaf (*Perilla frutescens* var. *acuta*).

[2)]Standard error of mean (n=12), [3)](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 ($P<0.05$).

4.3.4. Sensory evaluation

The scores of color were not significantly different among all treatments during all storage periods (Table 13). The flavor score was the highest in ERP 0 and the lowest in ERP 4 at 0 and 4 days of storage ($P<0.05$). The flavor score of ERP 2 showed no significant difference compared with that of non-injected at 0 and 4 days of storage. However, the flavor scores were not significantly different among all treatment after storage for 7 day. In taste, the scores were similar among non-injected, ERP 2, and ERP 4, while ERP 0 received the highest score during all storage periods. The odor and off-taste scores of ERP 4 were the highest among treatments during all storage periods. However, no significant differences of the odor and off-taste scores were found among non-injected, ERP 0, and ERP 2 at 0 and 7 days of storage. Non-injected showed the lowest score of tenderness during all storage periods. There were no significant differences of tenderness scores among pork loins with brine injection (ERP 0, 2, and 4) during all storage periods. In overall acceptability, ERP 0 and ERP 4 received the highest and lowest scores, respectively. However, ERP 2 showed no different scores compared with non-injected at 0 and 4 days of storage. In addition, no significant differences of overall acceptability scores were found among non-injected, ERP 0, and ERP 2 after storage for 7 day.

The high scores of sensorial properties in ERP 0 may be caused by added salt and phosphate because salt improves flavor and taste by serving as a contributor (Tarté, 2009). Moreover, Robbins *et al.*, (2003) reported that addition of tripolyphosphate to beef made increase in flavor and sensory properties. However, in the ERP 2 and ERP 4, odor and off-taste scores were

relatively high due to the characteristic flavor of the red perilla leaf (*Perilla frutescens* var. *acuta*). Mo *et al*. (1999) reported that the food had a lower sensory score due to unique flavor of red perilla leaf (*Perilla frutescens* var. *acuta*) when extract was added to food. The brine injection into pork loins (ERP 0, 2, and 4) resulted in the increase of the tenderness which is due to the influence of phosphate and salt ($P<0.05$). Lawrence *et al.* (2004) reported that the tenderness was increase by injecting phosphate and salt into beef.

Table 13. Sensory analysis of pork loin added different concentration of extract of red perilla leaf (*Perilla frutescens* var. *acuta*)

Traits	Storage days	Non-injected	ERP[1]			SEM[2]
			0	2	4	
Color	0	4.76	4.92	4.56	4.48	0.202
	4	5.15	5.37	4.56	4.70	0.265
	7	4.90	4.86	5.24	4.83	0.307
Flavor	0	5.20^{ab}	5.24^{a}	4.32^{bc}	3.60^{c}	0.246
	4	5.22^{ab}	5.48^{a}	4.90^{bc}	4.07^{c}	0.263
	7	5.45	5.69	5.86	4.93	0.285
Taste	0	5.00^{ab}	6.12^{a}	4.44^{b}	3.88^{b}	0.313
	4	5.48^{ab}	5.81^{a}	4.52^{b}	4.41^{b}	0.296
	7	5.45^{ab}	6.24^{a}	5.55^{ab}	4.48^{b}	0.314
Odor	0	2.04^{b}	1.92^{b}	2.72^{b}	4.96^{a}	0.419
	4	2.00^{b}	1.74^{b}	3.70^{a}	4.00^{a}	0.340
	7	2.31^{b}	2.31^{b}	3.28^{ab}	4.38^{a}	0.392
Off-taste	0	2.04^{b}	1.74^{b}	2.92^{b}	5.00^{a}	0.455
	4	1.80^{b}	1.44^{b}	3.70^{a}	4.30^{a}	0.361
	7	2.03^{b}	2.52^{b}	3.07^{b}	4.69^{a}	0.368
Tenderness	0	4.16^{b}	5.48^{a}	4.87^{ab}	4.68^{ab}	0.340
	4	3.52^{b}	5.15^{a}	5.44^{a}	5.78^{a}	0.311
	7	4.00^{b}	5.89^{a}	4.76^{ab}	5.10^{ab}	0.322
Overall acceptability	0	5.00^{b}	6.16^{a}	4.28^{bc}	3.88^{c}	0.289
	4	5.08^{ab}	5.93^{a}	4.50^{b}	4.19^{b}	0.279
	7	5.10^{ab}	6.07^{a}	5.17^{ab}	4.50^{b}	0.302

[1)]ERP, Extract of red perilla leaf (*Perilla frutescens* var. *acuta*).

[2)]Standard error of mean (n=12).

[a-c]Different letters within same row differ significantly ($P<0.05$).

5. Conclusion

ERP contained the highest phenolic content at a concentration of 71.76 mg GAE/g and showed the highest DPPH radical scavenging activity and reducing power compared with those of extracts derived from tomato, broccoli, and burdock. Although the instrumental color (L^*, a^*, and b^*-values) of raw and cooked pork loin was influenced by injection brine containing ERP, the color scores in sensory evaluation were not different among non-injected pork loin, ERP 0, ERP 2, and ERP 4. The lipid oxidation of cooked pork loin was effectively inhibited by brine injection containing ERP. The sensorial properties of pork loin such as flavor, odor, and off-flavor were adversely effected by ERP. However, the tenderness of pork loin was improved with brine injection without ERP or with ERP. In addition, the brine injection containing ERP into pork loins at a level equivalent 2 g/kg of pork loins' weight had no adverse effects on overall acceptability compared with non-injected. Therefore, the present study determined that the enhanced pork loin with improvement of oxidation stability can be produced by using ERP as natural antioxidant.

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V. Summary in Korea

최근 소비자의 육류에 대한 기호가 양적인 측면 보다는 연도, 다즙성 등의 품질적인 측면으로 전환되고 있으며 이에 따라 식육 산업에서는 고품질 식육을 생산하기 위하여 수년간 노력해왔다. 식육의 품질은 소비자가 식육을 구매할 시 영향을 미치는 주요 요소이며 보관 중에 품질 저하가 많이 발생한다. 식육 품질을 유지하고 향상시키기 위하여 포장 및 가공과 같은 다양한 방법을 사용할 수 있다.

그 중 하나로 최근 최소가공육(Enhanced meat)에 대한 관심이 증가하고 있다. 최소가공육이란 식육의 외관은 신선육과 동일하게 유지하되 소금, 인산염, 항산화제 등의 첨가제를 주입하여 품질을 향상시킨 것이다.

식육 품질에 영향을 미치는 여러 요인들 있으며 그 중 하나인 지방산화는 식육에서 품질 저하를 일으키는 주된 원인이다. 이는 식육의 변색, 풍미 저하, 저장 기간 감소뿐 아니라 독성 화합물 형성으로 영양적 손실까지 초래하므로 식육에서 지방산화를 막기 위한 노력이 많이 이루어지고 있다.

따라서 본 연구에서는 천연 항산화 물질을 돈육 등심에 주입하여 산화 안정성이 향상된 최소가공 돈육 등심을 개발하고 그에 따라, 천연 항산화 물질 선발(실험 I), 주입 최적 농도 설정(실험 II), 천연 항산화제를 돈육 등심에 주입하여 품질 평가(실험 III)로 나누어 연구를 진행 하였다.

실험 I 은 천연 항산화 물질 선발을 위해 폴리페놀이 다량 함유되어 있다고 알려진 천연 물질 4 가지(토마토, 브로콜리, 우엉, 자소엽)을 선정하였다. 4 가지 물질을 열수 추출 후 동결 건조를 통해 파우더를 제조하였고, 총 페놀 화합물 함량, DPPH 라디칼 소거능 및 환원력을 통해 항산화능을 측정하였다. 총 페놀 화합물

함량은 자소엽 추출물(71.76 ± 2.112 mg GAE/g)이 가장 높았고, DPPH 라디칼 소거능과 환원력을 측정한 결과, 자소엽 추출물이 가장 낮은 EC_{50} 를 보여 가장 높은 항산화력을 확인하였다. 따라서 본 실험 결과를 바탕으로 천연 항산화제로서 자소엽을 선정하였다.

실험 II 는 자소엽 추출물 적정 주입 농도 설정을 위해 돈육 패티에 자소엽 추출물 0, 2, 4, 6, 8 및 10 g/kg 를 첨가하여 항산화능을 측정하였다. 그 결과, 자소엽 추출물을 첨가한 돈육 패티에서 자소엽 추출물을 첨가하지 않은 돈육 패티보다 유의적으로 지방 산화의 진행이 감소된 것을 확인하였다. 그러나, 자소엽 추출물 2, 4, 6, 8 및 10 g/kg 첨가군 간의 유의적인 차이는 나타나지 않았다. 결과적으로 2 g/kg 과 4 g/kg 을 적정 주입 농도로 선택하였다.

실험 III 는 실험 I 과 실험 II 의 결과를 바탕으로 돈육 등심에 자소엽 추출물 2 g/kg 과 4 g/kg 을 주입하여 품질을 측정 하였다. 실험군은 1) 대조군(첨가제 무첨가군), 2) 소금 2 g/kg, 인산염 3 g/kg, 자소엽 추출물 0 g/kg 첨가군, 3) 소금 2 g/kg, 인산염 3 g/kg, 자소엽 추출물 2 g/kg 첨가군 및 4) 소금 2 g/kg, 인산염 3 g/kg, 자소엽 추출물 4 g/kg 첨가군으로 진행하였다. pH 와 가열 감량은 모든 처리간에 유의적인 차이가 나타나지 않았다. 저장 4 일차에 자소엽 추출물 2 g/kg 과 4 g/kg 첨가군은 대조군과 자소엽 추출물 0 g/kg 첨가군에 비하여 지방 산화의 진행이 감소되었으며 자소엽 추출물 2 g/kg 과 4 g/kg 첨가군은 대조군과 자소엽 추출물 0 g/kg 첨가군보다 명도(lightness, L^*)가 낮고, 적색도(redness, a^*)가 높아지는 것을 확인하였다($P<0.05$). 관능평가 결과를 볼 때 모든 저장 기간 동안 색의 유의적 차이가 나타나지 않았다. 반면, 자소엽 추출물 4 g/kg 첨가 시 이취와 이미에서 가장 높은 점수를 보였다. 이로 인해, 종합적 기호도는 자소엽 추출물 0 g/kg 첨가군에서 가장 높고, 자소엽 추출물 4 g/kg 첨가군에서 가장

낮은 점수를 보였다. 그러나, 저장 7 일 차에서는 대조군, 자소엽 추출물 0 g/kg 첨가군, 자소엽 추출물 2g/kg 첨가군 간의 유의적 차이가 나타나지 않았다.

최종적으로, 자소엽 추출물 2 g/kg 을 천연항산화제로 이용하여 돈육 등심에 주입함으로써 관능적으로 부정적인 영향이 없고, 산화 안정성이 증진된 최소가공 돈육 등심 생산이 가능할 것으로 보인다.