

## Supplementation of Pork Patties with Bovine Plasma Protein Hydrolysates Augments Antioxidant Properties and Improves Quality

Hyun-Woo Seo<sup>1</sup>, Jin-Kyu Seo, and Han-Sul Yang\*

*Division of Applied Life Science (BK21 plus) · Institute of Agriculture and Life Science,  
Gyeongsang National University, Jinju 52828, Korea*

<sup>1</sup>*National Institute of Animal Science, RDA, Wanju 55365, Korea*

### Abstract

This study investigated the effects of bovine plasma protein (PP) hydrolysates on the antioxidant and quality properties of pork patties during storage. Pork patties were divided into 4 groups: without butylated hydroxytoluene (BHT) and PP hydrolysates (control), 0.02% BHT (T1), 1% PP hydrolysates (T2), and 2% PP hydrolysates (T3). Pork patty supplemented with PP hydrolysates had higher pH values and lower weight loss during cooking than the control patties. Results showed that lightness and hardness both decreased upon the addition of PP hydrolysates. All samples containing BHT and PP hydrolysates had reduced TBARS and peroxide values during storage. In particular, 2% PP hydrolysates were more effective in delaying lipid oxidation than were the other treatments. It was concluded that treatment with 2% PP hydrolysates can enhance the acceptance of pork patty.

**Keywords:** bovine plasma, protein hydrolysates, pork patty, antioxidant activity, quality properties

*Received December 18, 2015; Revised February 4, 2016; Accepted February 4, 2016*

### Introduction

Lipid oxidation is a key factor in the deterioration of meat and meat products during processing and storage, leading to undesirable changes in color, flavor, texture, and nutritional profile. The extent and speed of oxidation depends on several factors, such as temperature, the presence of prooxidants and antioxidants, and the molecular nature of the lipids in the products (Frankel, 1985; Park *et al.*, 2012). Antioxidants have been widely used to prevent the oxidative process in meat and meat products (Jung *et al.*, 2012). The use of antioxidants is an effective method of preventing the generation of lipid oxidation products while maintaining nutritional quality and extending shelf-life.

The most common synthetic antioxidants used in the meat industry are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Both BHA and BHT have been widely used for many years to delay lipid oxidation and extend the shelf-life of meat and meat products (Nuñez de Gonzalez *et al.*, 2008). However, concerns

about the long-term safety and negative consumer perception of synthetic antioxidants have led to an increasing demand for the use of natural antioxidants in meat and meat products (Ahn *et al.*, 2002). It has been reported that some protein and enzymatic hydrolysates of meat and meat by-products are able to exert antioxidant activity in food products (Li *et al.*, 2007). In line with this, the demand for peptides or proteins as antioxidants in foods is increasing due to their low cost, safety, and high nutritional values (Hattori *et al.*, 1998).

In recent years, research has focused on the generation of peptides from food sources (Daoud *et al.*, 2005), which have been shown to exert antimicrobial, antihypertensive, and antioxidant effects. Peptides with antioxidative activity have been identified from enzymatic hydrolysates of egg albumin (Tsuge *et al.*, 1991), casein (Rival *et al.*, 2001), whey protein (Peña-Ramos *et al.*, 2004), gelatin (Kim *et al.*, 2001), and myofibrillar protein (Saiga *et al.*, 2003).

Plasma protein can be used in both the feed and food industries owing to its good nutritional value and excellent functional properties (Tybor *et al.*, 1975). Plasma protein hydrolysates, such as porcine plasma (Liu *et al.*, 2009) and bovine plasma (Salgado *et al.*, 2011), have been shown to possess antioxidant activity. However, hydrolyzed plasma protein has not been used as a functional food ingre-

\*Corresponding author: Han-Sul Yang, Division of Applied Life Science Graduate School, Gyeongsang National University, Jinju 52828, Korea. Tel: +82-55-772-1948, Fax: +82-55-772-1949, E-mail: hsyang@gnu.ac.kr

dient nor has it been evaluated as a potential antioxidant for food quality preservation. Also, there is little information regarding the functional peptides generated and how to establish antioxidant activity in meat-based by-products such as bovine plasma protein.

The objective of the present study was to determine the effectiveness of bovine plasma protein hydrolysates in preventing lipid oxidation in fresh pork patty. Also, the influence of the hydrolyzed plasma protein on other quality traits of the pork patties such as their color, texture, and sensory properties was investigated.

## Materials and Methods

### Preparation of bovine plasma protein hydrolysates

The plasma protein (PP) hydrolysates were prepared according to the method described by Liu *et al.* (2009). Cattle blood was anticoagulated by adding 0.5 N ethylenediaminetetraacetic acid (EDTA) in a 1:9 (v/v) proportion. This blood was immediately placed on ice and transported to the laboratory within 30 min. Samples were centrifuged (SUPRA 25K, Hanil Science, Korea) at 14,000 g for 15 min at 4°C. The plasma was then freeze-dried (Clean van 8B Freeze-Dryer, BioTron, Inc., Korea), pulverized, and stored in sealed bags at 4°C.

Bovine PP solution [5% w/v 10 mM sodium phosphate buffer (pH 7.0)] was heat treated (90°C, 5 min) and then hydrolyzed with Alcalase, with an enzyme to substrate ratio (E/S) of 2:100 (g/g). The pH of the bovine PP solution was adjusted to the optimal value for Alcalase (pH 8.32) before hydrolysis was initiated, and it was readjusted to the optimal value every 15 min during hydrolysis with 1 M NaOH. The hydrolysates were produced by setting the hydrolyzation time to 338 min and the temperature to 54°C. After hydrolysis, the pH of the solution was brought to 7.0 and the solution was then heated at 95°C for 5 min to inactivate the enzyme.

The degree of hydrolysis (DH) was determined by assaying free amino groups with 2, 4, 6-trinitrobenzenesulfonic acid (TNBS) according to Alder-Nissen (1986). The free amino content in samples was expressed as leucine amino equivalents, based on the equation of the leucine standard curve generated. The DH of hydrolyzed PP was calculated as  $[(h_s - h_0) / (h_t - h_0)] \times 100$  (DH = 18.8%, respectively).

Here  $h_s$  and  $h_0$  represent respectively the amino concentrations of hydrolyzed and non-hydrolyzed PP and  $h_t$  represents the total amino concentration of PP, as measured by completely hydrolyzing the PP with 6 N HCl. The hydrolysates were freeze-dried (Clean van 8B Freeze-Dryer,

BioTron, Inc., Korea), pulverized, and stored in sealed bags at 4°C.

### Preparation of pork patty

A total of nine pigs (Landrace × Yorkshire × Duroc; 100 ± 5 kg) were randomly selected at a commercial slaughter plant. Pork *longissimus dorsi* muscles (pH 5.54-5.57) were obtained at 48 h post-slaughter from a local market on three different processing days. Three replications of six pork loins (3 pigs) per replicate were used for each treatment. Each loin was trimmed of any visible fat and connective tissues, and each replication was ground separately through a 3-mm plate, twice. In each replication, four pork patty formulations, all with 1.5% (w/w) added NaCl, were prepared: (C) control (without the addition of BHT and PP hydrolysates); (T1) 0.02% BHT; (T2) 1% PP hydrolysates; (T3) 2% PP hydrolysates. For each formulation treatment, the mixture was prepared by blending for 5 min with a Kitchen Aid mixer (5K5SS, Kitchen Aid, USA). Duplicate patties of 100 g each were shaped by hand into approximately 10 cm (diameter) × 1 cm (thickness) rounds. The patties were placed in styrene foam trays and wrapped in an oxygen permeable poly (vinyl chloride) film, stored at 4°C for 1, 4 and 7 d of storage.

The rest of the patties were cooked in an electric oven at 175°C to an internal temperature of 75°C and used for sensory analysis. All cooked patties were vacuum-packaged immediately after cooking to minimize oxidative changes during handling before the sensory test. The cooked meats were tested for sensory test without storage.

### Proximate analysis

The moisture (method 920.36), crude protein (method 984.13), crude fat (method 991.36), and crude ash (method 938.08) contents were determined according to Association of Official Analytical Chemists (AOAC, 2000).

### pH measurement

The pH was measured in triplicate using a digital pH meter (MP230, Mettler Toledo, Switzerland). Approximately 3 g of patty sample was added to distilled water (27 mL). A slurry was then made using a homogenizer (ULTRA TURRAX T25D, IKA, Germany) and the pH was measured. The pH meter was calibrated daily with standard buffers of pH 4.0 and 7.0 at 25°C.

### Cooking loss

Pork patties were cooked on a heated metal plate (170°C)

by flipping every 2 min until the patties' internal temperature reached 73°C. The weight loss due to cooking was determined for each treatment replication combination. Weights of uncooked and cooked patties were recorded (Boles and Swan, 1996). The cooking loss was calculated as follows:

$$\text{Cooking loss (\%)} = \frac{\text{cooked weight}}{\text{uncooked weight}} \times 100$$

### Color

Color was measured using a Labscan Spectrophotometer (Hunter Associates Laboratory, Inc., USA) that had been calibrated against white and black reference tiles covered with the same film as those used for patty samples. CIE  $L^*$  (Lightness),  $a^*$  (redness),  $b^*$  (yellowness) values were obtained using illuminant A (light source). Area view and port size were 0.64 and 1.02 cm, and observer angle was 10°. An average value from three random locations of sample surface was used for statistical analysis.

### Texture profile analysis (TPA)

TPA was performed using an Instron University Testing Machine (Model 3343) with a 49 N load cell. Prior to testing, cooked pork patties were equilibrated to room temperature for 30 min and were cut into 2.5 cm diameter samples. The samples were compressed twice at a cross-head speed of 100 mm/min to 70% of their original height using a 3-in. diameter cylindrical plate. The textural parameters of hardness, cohesiveness, springiness, and gumminess were calculated.

### 2-thiobarbituric acid-reactive substance (TBARS)

Lipid oxidation was determined using a TBARS method (Buege and Aust, 1978). The amounts of TBARS were expressed as mg of malondialdehyde (MDA) per kg of sample.

### Peroxide values (PV)

The PV of pork patties were measured according to the AOCS standard procedure (1993). Specifically, patties were finely chopped by blending in a microwaving blender for exactly 30 s. A 5 g sample was then mixed with 25 mL of acetic acid-chloroform solution (3:2). The slurry was gently swirled to extract lipid and 1 mL saturated potassium iodine solution was then added. After reaction for 1 min with occasional shaking, 30 mL of distilled H<sub>2</sub>O and 1 mL of 0.1% starch solution were added. The solution was titrated with 0.01 N sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>)

until the intense blue color disappeared. A control blank (without meat sample) was also analyzed. The PV was calculated as

$$\text{PV (mequiv/kg)} = \frac{[(S - B) \times 1000 \times N]}{W}$$

where  $S$  and  $B$  are the volume (mL) of sodium thiosulfate solution consumed by the sample and by the blank, respectively,  $N$  is the concentration (N) of sodium thiosulfate solution, and  $W$  is the sample weight (g).

### Sensory evaluation

Pork patty samples from each treatment were evaluated by an 8-member trained expert descriptive attribute sensory panel in the Gyeongsang National University. Recruitment, selection, and training of panelists were performed according to the sensory evaluation procedure (Meilgaard, 1999). Eight panelists were screened from 12 potential panelists using a basic taste identification test and were trained with commercial pork patty products for 2 wk to familiarize them with the product characteristics planned to be evaluated. Panelists were given samples representing anchor points for each attribute, and training sessions using pork patties without antioxidant (control) and pork patties with antioxidant (BHT and 1%, 2% PP). The panelists were trained using a 5-point scale ("5 extremely intense" and "1 slightly intense") for color, cooked pork flavor, antioxidant flavor and texture attributes (hardness and juiciness).

Panelists evaluated the color, flavor, off-flavor, juiciness, tenderness, and overall acceptability of the samples using a 9-point hedonic, where 1 was "dislike extremely" and 9 was "like extremely" as described by Meilgaard *et al.* (1999). Four samples were provided to each panelist per session (3 sessions per each treatment). The samples were placed in glass containers (Pyrex, Belgium) with plastic covers before the sensory test.

### Statistical analysis

The experiment had three replications. Data was analyzed by the procedures of generalized linear model (GLM) of SAS (2014) Duncan's multiple range test was used to compare the mean values of treatments. Mean values and standard error of the means (SEM) were reported. Differences in sensory values were compared using the Tukey's significant differences. For sensory data, mean values and standard deviations were reported. Statistical significance for all comparisons was made at  $p < 0.05$ .

## Results and Discussion

### Proximate analysis

The proximate compositions of the pork patties in each group are shown in Table 1. The T1, T2, and T3 pork patties showed no significant difference in crude protein and crude fat contents compared to the control patties. However, the moisture content of the pork patty samples ranged from 62.18% to 64.52%, with The T3 patties showing a higher moisture content than the patties in the other treatment groups ( $p < 0.05$ ). This finding suggests that the evaluation of physical properties and sensory quality in the T3 patties could potentially be biased by different moisture contents. It was also found that the ash content of pork patties was increased by the addition of BHT and hydrolyzed PP ( $p < 0.05$ ).

### pH and cooking loss

Changes in pH and cooking weight loss in pork patties during storage at 4°C are presented in Table 2. During storage, the control and T1 pork patty did not show significant changes in pH during 7 d of storage. The T2 patties had a lower pH at the start, but this increased after 4 d and then maintained by the end of storage ( $p < 0.05$ ). The T3 patties had the highest pH values throughout the storage period. Thus, in comparison to the control patties, the patties containing PP hydrolysates had higher pH values over the 7 d storage period ( $p < 0.05$ ). The relationship between pH and muscle protein denaturation has previously been reported by Bendall and Wismer-Pederson (1962). Further, the effectiveness of antioxidants is dependent on pH. It was previously shown that the pH value of chicken meat was increased with garlic supplementation (Kim *et al.*, 2009). Thus, similar to previous studies, the present experiment has shown that the pH value of pork patty can be altered through the addition of hydrolyzed PP.

The weight loss during cooking was significantly reduced in the T1, T2, and T3 patties compared with the control patties over the 7 d storage period ( $p < 0.05$ ). Hydrolyzed PP was particularly effective, reducing the weight loss of control patties (23.78%) to 15.41% and 11.30% ( $p < 0.05$ ) in T2 and T3 patties, respectively. Furthermore, higher concentrations of added PP hydrolysates tended to decrease percent cooking loss. This variation in weight loss during cooking of pork patties was expected as a positive relationship between weight loss and muscle pH has been previously demonstrated (Joo *et al.*, 1999). In line, an increase in pH in the antioxidant-treated samples was associated with reduced weight loss during cooking in the

**Table 1. Proximate composition (%) of pork patties**

Treatments <sup>1)</sup>	Moisture	Crude protein	Crude fat	Ash
C	63.14 <sup>B</sup>	20.42	10.61	1.33 <sup>B</sup>
T1	62.81 <sup>B</sup>	21.77	10.70	1.67 <sup>A</sup>
T2	63.21 <sup>B</sup>	20.98	11.18	1.65 <sup>A</sup>
T3	64.52 <sup>A</sup>	20.16	10.62	1.76 <sup>A</sup>
SEM	0.62	0.03	0.50	0.01

SEM: standard error of the means ( $n=3$ ).

<sup>A,B</sup>Means differ significantly ( $p < 0.05$ ) between treatment groups within a column.

<sup>1</sup>C, control without the addition of BHT or protein hydrolysates; T1, 0.02% BHT; T2, 1% plasma protein hydrolysates; T3, 2% plasma protein hydrolysates.

**Table 2. pH, cooking weight loss (%), and color (CIE values) in pork patties during storage at 4°C**

Treatments <sup>1)</sup>	Storage periods (d)				
	1	4	7	SEM	
pH	C	5.75 <sup>B</sup>	5.65 <sup>C</sup>	5.65 <sup>C</sup>	0.01
	T1	5.62 <sup>C</sup>	5.62 <sup>C</sup>	5.62 <sup>C</sup>	0.00
	T2	5.60 <sup>Cc</sup>	5.75 <sup>Ba</sup>	5.71 <sup>Bb</sup>	0.00
	T3	5.85 <sup>Aab</sup>	5.87 <sup>Aa</sup>	5.81 <sup>Ab</sup>	0.00
	SEM	0.01	0.01	0.00	
Cooking loss (%)	C	23.78 <sup>Aa</sup>	23.61 <sup>Aa</sup>	20.98 <sup>Ab</sup>	0.81
	T1	21.51 <sup>B</sup>	22.59 <sup>A</sup>	21.23 <sup>A</sup>	1.50
	T2	15.41 <sup>C</sup>	16.77 <sup>B</sup>	15.52 <sup>B</sup>	0.95
	T3	11.30 <sup>D</sup>	12.22 <sup>C</sup>	11.67 <sup>C</sup>	0.63
	SEM	1.26	0.78	1.03	
<i>L</i> * (Lightness)	C	56.06 <sup>A</sup>	57.17 <sup>B</sup>	56.82 <sup>AB</sup>	5.30
	T1	56.95 <sup>Ab</sup>	59.17 <sup>Aa</sup>	57.29 <sup>Ab</sup>	4.56
	T2	54.17 <sup>Bb</sup>	56.58 <sup>Bca</sup>	57.51 <sup>Aa</sup>	4.89
	T3	52.85 <sup>Bb</sup>	55.57 <sup>Ca</sup>	55.72 <sup>Ba</sup>	6.15
	SEM	5.04	5.25	5.40	
<i>a</i> * (Redness)	C	12.17 <sup>Aa</sup>	11.52 <sup>Ab</sup>	10.92 <sup>Ab</sup>	2.43
	T1	12.27 <sup>Aa</sup>	11.34 <sup>Ab</sup>	11.07 <sup>Ab</sup>	1.13
	T2	11.90 <sup>Aa</sup>	10.60 <sup>Bb</sup>	9.36 <sup>Bc</sup>	0.94
	T3	10.83 <sup>Ba</sup>	9.89 <sup>Cb</sup>	9.54 <sup>Bb</sup>	0.34
	SEM	2.02	0.81	0.81	
<i>b</i> * (Yellowness)	C	5.75 <sup>D</sup>	5.64 <sup>C</sup>	5.59 <sup>C</sup>	0.83
	T1	6.38 <sup>Ca</sup>	5.63 <sup>Cb</sup>	5.66 <sup>Cb</sup>	0.64
	T2	7.51 <sup>Ba</sup>	7.03 <sup>Bab</sup>	6.83 <sup>Bb</sup>	0.63
	T3	8.33 <sup>A</sup>	8.39 <sup>A</sup>	8.53 <sup>A</sup>	0.67
	SEM	0.73	0.84	0.51	

SEM: standard error of the means ( $n=3$ ).

<sup>A-D</sup>Means differ significantly ( $p < 0.05$ ) between treatment groups within a column.

<sup>a,b</sup>Means differ significantly ( $p < 0.05$ ) between storage times within a row.

<sup>1</sup>C, control without the addition of BHT or protein hydrolysates; T1, 0.02% BHT; T2, 1% plasma protein hydrolysates; T3, 2% plasma protein hydrolysates.

present study. Consistently, Peña-Ramos and Xiong (2003) also reported a reduction in weight loss during cooking in ground meat supplemented with soy and whey protein hydrolysates. A potential explanation for this relationship

was suggested in a previous study, in which it was found that hydrolysis led to dissociation of potato protein into subunits, thus producing additional polar and charged groups allowing for stronger protein-water interactions in meat (Wang and Xiong, 2005).

### Color

The changes in color pork patties during storage at 4°C are presented in Table 2. During storage, the control pork patty did not show significant changes in lightness ( $L^*$ ) during 7 d of storage. However, the  $L^*$  values increased during storage for 7 d in treatment groups. On day 1, T2 and T3 pork patties had lower  $L^*$  values than the patties in the other treatment groups ( $p < 0.05$ ), with the T3 patties showing the lowest  $L^*$  values across the 7 d storage period. Antioxidants such as  $\alpha$ -tocopherol and rosemary are effective in preventing meat discoloration. It is widely accepted that variations in muscle structure may affect the extent of denaturation of muscle protein, allowing for differences between pale and dark colored meats (Chen *et al.*, 1999). Further, the different results may be related to the different materials or processing methods employed. Beggs *et al.* (1997) have shown that the level of modified starch added to turkey frankfurters affected color values. Additionally, Nuñez de Gonzalez *et al.* (2008) reported that the color of pork sausage supplemented with dried plum is darker due to the original dark purple color of the plum.

Redness ( $a^*$ ) varied from 9.36 to 12.27 over the storage time period. All pork patty samples showed significant changes in  $a^*$  values during 7 d of storage at 4°C. Redness values were lower in the T2 and T3 pork patties compared to the control and T1 patties throughout the 7 d period ( $p < 0.05$ ). Yellowness ( $b^*$ ) values were higher in the T2 and T3 patties compared to the control and T1 patties, with the T3 patties showing the highest values across the 7 d storage period ( $p < 0.05$ ). The  $b^*$  values of the T2 and T3 pork patties were slightly higher compared to that of the T1 patties. Thus, the addition of PP hydrolysates changed the color attributes of the pork patties by decreasing lightness and redness and increasing yellowness.

### Texture profile analysis

The texture attributes of the pork patties are shown in Table 3. Hardness values were tended to lower for the T2 and T3 patties compared to the patties in the other treatment groups ( $p < 0.05$ ). Furthermore, on day 4, the patties containing PP hydrolysates (T2 and T3) showed significantly decreased hardness values compared to the patties in the control and T1 groups ( $p < 0.05$ ). Also, the cohesive-

**Table 3. Texture properties of pork patties during storage at 4°C**

Treatments <sup>1)</sup>	Storage periods (d)				
	1	4	7	SEM	
Hardness (N)	C	4.28 <sup>Ab</sup>	5.96 <sup>Aa</sup>	4.50 <sup>Ab</sup>	0.88
	T1	3.42 <sup>ABb</sup>	5.06 <sup>Aa</sup>	3.88 <sup>ABb</sup>	0.58
	T2	3.00 <sup>B</sup>	3.07 <sup>B</sup>	3.60 <sup>B</sup>	0.36
	T3	3.78 <sup>Aa</sup>	2.27 <sup>Bb</sup>	4.15 <sup>ABa</sup>	0.54
	SEM	0.40	0.11	0.35	
Cohesiveness	C	0.36 <sup>Bb</sup>	0.47 <sup>a</sup>	0.46 <sup>Bab</sup>	0.04
	T1	0.38 <sup>Bb</sup>	0.45 <sup>a</sup>	0.44 <sup>Ba</sup>	0.01
	T2	0.41 <sup>Bb</sup>	0.45 <sup>ab</sup>	0.49 <sup>Ba</sup>	0.03
	T3	0.56 <sup>A</sup>	0.50	0.60 <sup>A</sup>	0.07
	SEM	0.03	0.02	0.03	
Springiness	C	26.22 <sup>B</sup>	21.57	17.75 <sup>B</sup>	3.01
	T1	13.79 <sup>Cb</sup>	22.28 <sup>a</sup>	16.52 <sup>Bab</sup>	3.47
	T2	20.86 <sup>BC</sup>	21.61	27.17 <sup>B</sup>	1.03
	T3	50.81 <sup>Aa</sup>	20.75 <sup>b</sup>	53.64 <sup>Aa</sup>	4.05
	SEM	2.60	3.70	3.04	
Gumminess (N)	C	24.50 <sup>ABc</sup>	41.89 <sup>Aa</sup>	35.62 <sup>Ab</sup>	1.70
	T1	22.84 <sup>BCc</sup>	36.27 <sup>Aa</sup>	29.35 <sup>BCb</sup>	1.19
	T2	19.60 <sup>Cb</sup>	23.21 <sup>Bab</sup>	25.59 <sup>Ca</sup>	1.53
	T3	26.20 <sup>Ab</sup>	18.80 <sup>Bc</sup>	33.33 <sup>ABa</sup>	1.22
	SEM	0.73	2.48	1.17	

SEM: standard error of the means ( $n=3$ ).

<sup>A-C</sup>Means differ significantly ( $p < 0.05$ ) between treatment groups within a column.

<sup>a-c</sup>Means differ significantly ( $p < 0.05$ ) between storage times within a row.

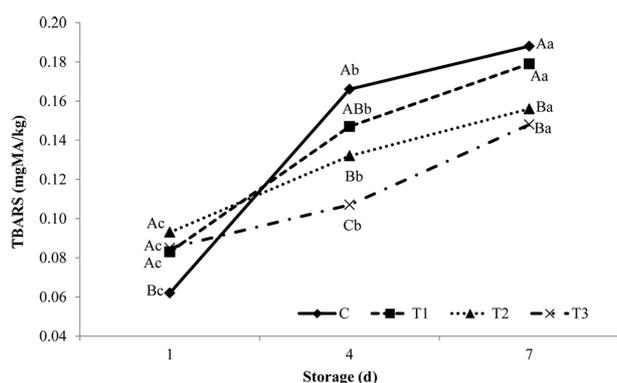
<sup>1)</sup>C, control without the addition of BHT or protein hydrolysates; T1, 0.02% BHT; T2, 1% plasma protein hydrolysates; T3, 2% plasma protein hydrolysates.

ness and springiness values were higher in the T3 patties compared to the patties in the other treatment groups throughout storage, except at 4 d ( $p < 0.05$ ). All treatments (T1, T2, and T3) had lower gumminess values over the 7 d storage period. Of the treated samples, gumminess values were lower in the T2 patties compared to the patties in the other treatment group over the 7 d period ( $p < 0.05$ ).

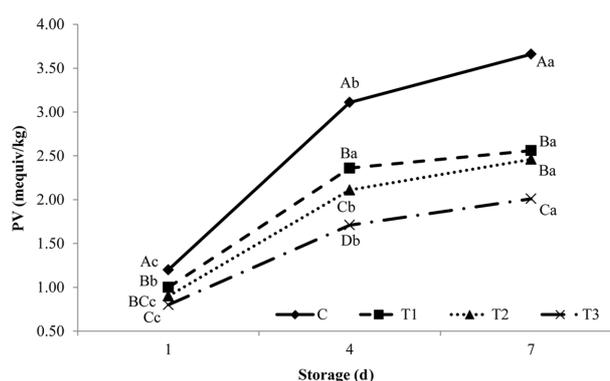
These results indicate that the addition of PP hydrolysates is useful in preparing a pork patty with softer textural properties. These results are consistent with those reported by other researchers, who demonstrated that the addition of hydrolyzed soy protein isolates improved textural properties by decreasing product hardness (Feng *et al.*, 2003). Yang *et al.* (2007) suggested that a decrease in the hardness of sausage by the addition of texture-modifying agents may be associated with the water binding properties of the ingredients.

### Inhibition of lipid oxidation in pork patties

The results of the lipid oxidation analysis of pork patties during storage at 4°C are shown in Figs. 1 and 2. The



**Fig. 1.** TBARS values in pork patties during storage period at 4°C. <sup>A-C</sup>Means differ significantly ( $p < 0.05$ ) between treatment groups. <sup>a-c</sup>Means differ significantly ( $p < 0.05$ ) between storage times within a treatment group. Control, without the addition of BHT or protein hydrolysates; T1, 0.02% BHT; T2, 1% plasma protein hydrolysates; T3, 2% plasma protein hydrolysates.



**Fig. 2.** Peroxide value (PV) of pork patties during storage at 4°C. <sup>A-C</sup>Means differ significantly ( $p < 0.05$ ) between treatment groups. <sup>a-c</sup>Means differ significantly ( $p < 0.05$ ) between storage times within a treatment group. Control, without the addition of BHT or protein hydrolysates; T1, 0.02% BHT; T2, 1% plasma protein hydrolysates; T3, 2% plasma protein hydrolysates.

TBARS assay is commonly used for detecting the decomposition products of peroxides, such as aldehydes (Angelo, 1996). In all treatment groups, the pork patties showed an increasing trend in TBARS as the storage period increased (Fig. 1). However, TBARS values were lower in the T2 and T3 patties compared to the patties in the other treatment groups throughout the 7 d period ( $p < 0.05$ ). Overall, both BHT and hydrolyzed PP inhibited TBARS formation in patties during storage. In particular, the addition of PP hydrolysates was more effective in decreasing TBARS values of the pork patties than was the addition of BHT after 4 d storage ( $p < 0.05$ ).

The peroxide value (PV) is used as a measure of primary oxidation products in meat and meat products. The progress of lipid oxidation as indicated by PV following the addition of BHT and PP hydrolysates to pork patties is shown in Fig. 2. In all treatment groups, the PV at day 7 had dramatically increased compared to day 1 ( $p < 0.05$ ). However, the PV was lower in the T1, T2, and T3 pork patties compared to the control patties throughout the 7 d period ( $p < 0.05$ ). Furthermore, the addition of PP hydrolysates was more effective in decreasing PV of pork patties than was the addition of BHT during storage ( $p < 0.05$ ). Also, the PV of pork patties treated with 1 or 2% hydrolyzed plasma protein (2.46 and 2.01 mequiv/kg) at the end of storage represented 32.78% and 45.08% oxidation inhibitions ( $p < 0.05$ ), respectively, when compared with that of control patties (3.66 mequiv/kg).

The antioxidative effect of plasma protein hydrolysates on pork patties in this study was remarkable. In concor-

dance, Wang and Xiong (2005) reported that hydrolyzed potato proteins inhibited PV and TBARS formation in cooked beef patties during storage. Additionally, several previous studies have indicated that hydrolyzed whey and soy protein (Peña-Ramos and Xiong, 2003) mechanically deboned chicken (Jin *et al.*, 2014) egg-yolk protein (Sakanaka *et al.*, 2004), and porcine haemoglobin (Chang *et al.*, 2007) were antioxidative in meat products. Therefore, adding PP hydrolysates to fresh pork patties affords enhanced antioxidant activity and protection against lipid oxidation.

### Sensory evaluation

Ultimately, meat product quality is defined in terms of consumer acceptability which includes color, tenderness, juiciness, tenderness and flavor (Robbins *et al.*, 2003). In addition, appearance characteristics have a significant impact on consumer expectations (Brewer and Novakofski, 2008). The results of the sensory evaluation of pork patties are shown in Table 4. There were no significant differences in flavor and texture scores ( $p > 0.05$ ) between any of the treatment groups. The off-flavor scores in pork patties with added BHT and PP hydrolysates were higher than those in the control patties ( $p < 0.05$ ). Addition of crude wheat gluten hydrolysate in pork patties did not adversely affect the color, smell, taste, texture, and overall acceptability of cooked pork (Park *et al.*, 2012). Further, increasing levels of PP hydrolysates tended to increase the off-flavor scores. However, sensory scores for color and juiciness were higher in the patties with added PP hydrolysates than in the control patties. Some authors sug-

**Table 4. Sensory evaluations of pork patties (Tukey's HSD 5%)**

Treatments <sup>1)</sup>	Color	Flavor	Off-flavor	Juiciness	Texture	Overall acceptability
C	3.75±0.97 <sup>B</sup>	4.92±1.16	2.17±0.58 <sup>C</sup>	4.33±1.30 <sup>C</sup>	4.58±1.16	4.82±0.75 <sup>B</sup>
T1	4.27±0.90 <sup>AB</sup>	4.91±1.45	2.27±0.79 <sup>BC</sup>	4.73±1.42 <sup>C</sup>	4.64±1.12	5.00±1.05 <sup>B</sup>
T2	4.55±1.04 <sup>AB</sup>	5.00±1.48	2.82±0.75 <sup>AB</sup>	5.64±1.30 <sup>AB</sup>	4.82±0.98	5.70±0.95 <sup>AB</sup>
T3	5.00±1.49 <sup>A</sup>	4.90±1.45	3.20±0.63 <sup>A</sup>	6.10±0.88 <sup>A</sup>	5.00±1.15	6.22±1.30 <sup>A</sup>

Data are means±standard errors.

<sup>A-C</sup>Means differ significantly ( $p<0.05$ ) between treatment groups within a column. ( $n=8$ ).

<sup>1</sup>C, control without the addition of BHT or protein hydrolysates; T1, 0.02% BHT; T2, 1% plasma protein hydrolysates; T3, 2% plasma protein hydrolysates.

Based on a 9-point intensity scale (1 = dislike extremely or extremely light/bland/tough/dry; and 9 = like extremely or extremely dark/intense/tender/juice).

gested that juiciness scores increased primarily as a result of increased moisture (Gök *et al.*, 2011; Yi *et al.*, 2012). The addition of 2% PP hydrolysates resulted in significantly higher color and juiciness scores compared to all other treatments ( $p<0.05$ ). The overall acceptability scores ranged from 4.82 to 6.22, with maximum acceptability obtained in the patties containing 2% PP hydrolysates.

### Conclusion

This study concluded that PP hydrolysates provide antioxidant and quality benefits to fresh pork patties during refrigerated storage. Pork patties supplemented with PP hydrolysates have higher pH, and lower hardness and lightness values than control patties. Hydrolyzed PP products at the 1 or 2% level were able to not only reduce the weight loss during cooking but also to suppress lipid oxidation in pork patties during refrigerated storage. The addition of PP hydrolysates was more effective than the addition of BHT in decreasing peroxide and TBARS values of pork patties during storage. These results indicate that the PP hydrolysates were as effective as, if not superior to, BHT in retarding lipid oxidation in pork patty. Furthermore, the addition of 2% PP hydrolysates resulted in significantly higher color, juiciness, and overall acceptability scores compared to all other treatments ( $p<0.05$ ). Overall, the combination of the antioxidant effect together with the enhanced quality properties highlight the potential for PP hydrolysates to be utilized as attractive natural ingredients in processed muscle foods.

### Acknowledgements

This research was supported (Project No.315017-05-1-SB150) by IPET (Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and

Fisheries), Ministry of Agriculture, Food and Rural Affairs.

### References

- Ahn, J., Gruen, I. U., and Fernando, L. N. (2002) Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *J. Food Sci.* **67**, 1364-1369.
- Alder-Nissen, J. (1986) Some fundamental aspects of food protein hydrolysis. *Enzymatic hydrolysis of food proteins*. New York: Elsevier Applied Science Publishers, pp. 172-200.
- Angelo, A. J. S. (1996) Lipid oxidation in foods. *Crit. Rev. Food Sci. Nutr.* **36**, 175-224.
- AOAC (2000) *Official methods of analysis*. (17th ed.). Gaithersburg, MD: Association of Official Analytical Chemists.
- AOCS Official Method (1993) AOCS Official Method Cd 8-53. In F. Gunstone (Ed.), *Official methods and recommended practices of the American Oil Chemists' Society Method Cd 8-53*. Peroxide value acetic-chloroform method (4th ed.). AOCS Press, Champaign, IL.
- Beggs, K. L. H., Bowers, J. A., and Brown, D. (1997) Sensory and physical characteristics of reduced-fat turkey frankfurters with modified cornstarch and water. *J. Food Sci.* **62**, 1240-1244.
- Bendall, J. R. and Wismer-Pedersen, J. (1962) Some properties of the fibrillary protein of normal and watery pork muscle. *J. Food Sci.* **27**, 144-159.
- Boles, J. A. and Swan, J. E. (1996) Effect of post-slaughter processing and freezing on the functionality of hot-boned meat from young bull. *Meat Sci.* **44**, 11-18.
- Brewer, M. S. and Novakofski, J. E. (2008) Consumer quality evaluation of aging of beef. *J. Food Sci.* **73**, 78-82.
- Buege, J. A. and Aust, S. D. (1978) Microsomal lipid peroxidation. *Methods Enzymol.* **52**, 302-310.
- Chang, C. Y., Wu, K. C., and Chiang, S. H. (2007) Antioxidant properties and protein compositions of porcine haemoglobin hydrolysates. *Food Chem.* **100**, 1537-1543.
- Chen, W., Jo, C., Lee, J. I., and Ahn, D. U. (1999) Lipid oxidation volatiles and color changes of irradiated pork patties as affected by antioxidants. *J. Food Sci.* **64**, 16-19.
- Daoud, R., Dubios, V., Bors-Dodita, L., Nedjar-Arroume, N.,

- Krier, F., and Chihib, N. E. (2005) New antibacterial peptide derived from bovine hemoglobin. *Peptides* **26**, 713-719.
14. Feng, J., Xiong, Y. L., and Mikel, W. B. (2003) Textural properties of pork frankfurters containing thermally/enzymatically modified soy proteins. *J. Food Sci.* **68**, 1220-1224.
  15. Frankel, E. N. (1985) Chemistry of autoxidation: Mechanism, products, and flavor significance. In Min DB and Smouse TH (Eds.), *Flavor chemistry of fats and oils*. American Oil Chemists' Society: Champaign, IL., pp. 1-34.
  16. Gök, V., Akkaya, L., Obuz, E., and Bulut, S. (2011) Effect of ground poppy seed as a fat replacer on meat hamburgers. *Meat Sci.* **89**, 400-404.
  17. Hattori, M., Yamaji-Tsukamoto, K., Kumagai, H., Feng, Y., and Takahashi, K. (1998) Antioxidative activity of solution elastin peptides. *J. Agric. Food Chem.* **46**, 2167-2170.
  18. Jin, S. K., Hwang, J. W., Moon, S. S., Choi, Y. J., Kim, G. D., Jung, E. Y., and Yang, H. S. (2014) The effects of mechanically deboned chicken hydrolysates on the characteristics of imitation crab stick. *Korean J. Food Sci. Ani. Resour.* **34**, 192-199.
  19. Joo, S. T., Kauffman, R. G., Kim, B. C., and Park, G. B. (1999) The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. *Meat Sci.* **52**, 291-297.
  20. Jung, E. Y., Yun, I. R., Go, G. W., Kim, G. D., Seo, H. W., Joo, S. T., and Yang, H. S. (2012) Effects of *radix puerariae* extracts on physicochemical and sensory quality of precooked pork sausage during cold storage. *LWT-Food Sci. Technol.* **46**, 556-562.
  21. Kim, S. K., Kim, Y. T., Byun, H. G., Nam, K. S., Joo, D. S., and Shahidi, F. (2001) Isolation and characterization of antioxidative peptides from gelation hydrolysate of Alaska Pollock skin. *J. Agric. Food Chem.* **49**, 1984-1989.
  22. Kim, Y. J., Jin, S. K., and Yang, H. S. (2009) Effect of dietary garlic bulb and husk on the physicochemical properties of chicken meat. *Poult. Sci.* **88**, 398-405.
  23. Li, B., Chen, F., Xang, X., Ji, B., and Wu, Y. (2007) Isolation and identification of antioxidative peptides from porcine collagen hydrolysate by consecutive chromatography and electrospray ionization-mass spectrometry. *Food Chem.* **102**, 1135-1143.
  24. Liu, Q., Kong, B., Jiang, L., Cui, X., and Liu, J. (2009) Free radical scavenging activity of porcine plasma protein hydrolysates determined by electron spin resonance spectrometer. *LWT-Food Sci. Technol.* **42**, 956-962.
  25. Meilgaard, M., Civille, G. V., and Carr, B. T. (1999) *Sensory evaluation techniques*. (3rd ed.). CRC Press: Boca Raton, FL.
  26. Nuñez de Gonzalez, M. T., Boleman, R. M., Miller, R. K., Keeton, J. T., and Rhee, K. S. (2008) Antioxidant properties of dried plum ingredients in raw and precooked pork sausage. *J. Food Sci.* **73**, H63-H71.
  27. Park, E. Y., Imaza, H., Matsumura, Y., Nakamura, Y., and Sato, K. (2012) Effects of peptide fraction with different isoelectric points from wheat gluten hydrolysates on lipid oxidation in pork meat patties. *J. Agric. Food Chem.* **60**, 7483-7488.
  28. Peña-Ramos, E. A. and Xiong, Y. L. (2003) Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Sci.* **64**, 259-263.
  29. Peña-Ramos, E. A., Xiong, Y. L., and Arteaga, G. E. (2004) Fractionation and characterization for antioxidant activity of hydrolyzed whey protein. *J. Sci. Food Agric.* **84**, 1908-1918.
  30. Rival, S. G., Boeriu, C. G., and Wichers, H. J. (2001) Casein and casein hydrolysates. 2. Antioxidative properties and relevance to lipoxygenase inhibition. *J. Agric. Food Chem.* **4**, 259-302.
  31. Robbins, K., Jensen, J., Ryan, K. J., Homco-Ryan, C., McKeith, F. K., and Brewer, M. S. (2003) Consumer attitudes towards beef and acceptability of enhanced beef. *Meat Sci.* **65**, 721-729.
  32. Saiga, A., Tananbe, S., and Nishimura, T. (2003) Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *J. Agric. Food Chem.* **51**, 3661-3667.
  33. Sakanaka, S., Tachibana, Y., Ishihara, N., and Juneja, L. R. (2004) Antioxidant activity of egg-yolk protein hydrolysates in a linoleic acid oxidation system. *Food Chem.* **86**, 99-103.
  34. Salgado, P. R., Fernández, G. B., Drago, S. R., and Mauri, A. N. (2011) Addition of bovine plasma hydrolysates improves the antioxidant properties of soybean and sunflower protein-based films. *Food Hydrocoll.* **25**, 1433-1440.
  35. SAS Institute (2014) *SAS User's Guide: Version 9.3*. Cary, NC: SAS Institute, Inc.
  36. Tsuge, N., Eikawa, Y., Nomura, Y., Yamamoto, M., and Sugisawa, K. (1991) Antioxidative activity of peptides prepared and enzymatic hydrolysis of egg-white albumin. *Nippon Nogei Kagaku Kaishi* **65**, 1635-1641.
  37. Tybor, P. T., Dill, W., and Landmann, W. A. (1975) Functional properties of proteins isolated from bovine by a continuous pilot process. *J. Food Sci.* **40**, 155-159.
  38. Wang, L. L. and Xiong, Y. L. (2005) Inhibition of lipid oxidation in cooked beef patties by hydrolyzed potato protein is related to its reducing and radical scavenging ability. *J. Agric. Food Chem.* **53**, 9186-9192.
  39. Yang, H. S., Choi, S. G., Jeon, J. T., Park, G. B., and Joo, S. T. (2007) Textural and sensory properties of low fat pork sausages with added hydrated oatmeal and tofu as texture-modifying agents. *Meat Sci.* **75**, 283-289.
  40. Yi, H. C., Cho, H., Hong, J. J., Ryu, R. K., Hwang, K. T., and Regenstein, J. M. (2012) Physicochemical and organoleptic characteristics of seasoned beef patties with added glutinous rice flour. *Meat Sci.* **92**, 464-468.