

## Effects of Pumpkin (*Cucurbita moschata* Duch.) Leaf Ethanolic Extracts on Lipid Oxidation and Microbial Activity in Refrigerated Raw Ground Pork

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This study was conducted to evaluate the antioxidant effects of pumpkin leaf extracted using a 50% ethanol on ground pork during storage. The pumpkin leaf extracts were added at concentrations of 0.05 (PE-0.05), 0.1 (PE-0.1), and 0.2% (PE-0.2) to ground pork, and 0.05% of ascorbic acid (As-0.05) was added as a control. Each sample was collected after 1, 4, 7, and 10 d of storage and the pH, total viable counts (TVC), conjugated dienes (CD), free fatty acids (FFA), and thiobarbituric reaction substance (TBARS) values were measured. The pH of the pork samples decreased until day 7, and then increased thereafter, except for the control and PE-0.05 sample. Lower CIE a\* values were observed for pork samples containing PE relative to As-0.05 at increasing storage time ( $p < 0.05$ ). The addition of PE decreased the TVC, CD, FFA and TBARS values levels in the ground pork when compared to the control during 10 d of storage. These results indicate that PE can produce notable effects on meat products, such as inhibiting lipid oxidation and discoloration.

**Key words:** pumpkin leaf, lipid oxidation stability, shelf-life stability, ground pork

### Introduction

Ground pork is widely used due to convenience in the food industry around the world. Ground pork is also used as an ingredient *Tteok-galbi*, which is a traditional Korean food, and in hamburger patties. However, grinding accelerates lipid oxidation through oxidative reactions. Hydroperoxides, which are the primary oxidation products, are formed and then decompose into low molecular weight compounds such as ketones, aldehydes, and hydroxides during lipid oxidation of meat (St. Angelo, 1996). These lipid oxidation products result in unacceptable color, reduced shelf-life, unacceptable odors, and sensory changes (Ladikos and Lougovois, 1990).

Autoxidation in meat can be effectively inhibited using synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) (Barlow, 1990). However, due to growing concerns about food safety, the use of synthetic antioxidants has decreased because of their potential genotoxicity and carcinogenicity (Gharavi *et al.*, 2007). Consumer interest

in safety has resulted in an increased utilization of natural antioxidants in meat products (Hinneburg *et al.*, 2006). The addition of plant materials can provide minerals, vitamins, phenolic compounds, and flavonoid compounds to meet these demands.

Pumpkin (*Cucurbita moschata* Duch.) is cultivated all over the world and belongs to the family Cucurbitaceae. Pumpkin leaf has been widely used as a major ingredient in rice wraps, soups, and fermented foods. Traditionally, pumpkin leaf has been used to treat health problems such as night blindness, burns, and xerotic keratitis due to its pharmacological activity (Cha, 2009). Pumpkin leaf contains abundant antioxidant compounds including carotenoids and tocopherols (Stevenson *et al.*, 2007), phenolic compounds, and trace elements (Glew *et al.*, 2006). Several studies have examined the antioxidant activities of pumpkin (Kwon *et al.*, 2007), pumpkin leaf (Cha, 2009), pumpkin seeds (Xanthopoulou *et al.*, 2009), pumpkin oil (Fruhwith *et al.*, 2003), and pumpkin seed flour (Parry *et al.*, 2008).

In this study, the antioxidant activity of a pumpkin leaf ethanolic extract was investigated in ground pork meat during chilled storage, as measured by instrumental color, total viable counts (TVC), conjugated dienes (CD), free fatty acids (FFA), and thiobarbituric acid reaction substance (TBARS) values and compared with ascorbic acid.

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In addition, the proximate and pH values of the ground pork meat containing pumpkin leaf ethanolic extracts were evaluated during 10 d of storage.

## Materials and Methods

### Preparation of pumpkin (*Cucurbita moschata* Duch.) leaf extracts

The pumpkin leaves were washed and cut to separate the leaves and stems. The leaves were cut into small pieces, dried in a hot air dryer (Enex-Co-600, Enex, Korea) at 50°C for 12 h, and powdered (35 mesh). The dried pumpkin leaf powder (10 g) was extracted with 200 mL of 50% ethanol overnight in a shaker (VS-8480, Vison Scientific, Korea) at room temperature. The extract was filtered through Whatman No. 1 filter paper and the solvent was removed using a vacuum evaporator (CCA-1110, Rikakikai, Japan) at 45°C. After evaporation of ethanol, the pumpkin leaves ethanolic extracts were dissolved in 50% ethanol (5%, v/w).

### Preparation of meat samples

Fresh pork hams and back fats were purchased from a pilot plant at Konkuk University, Korea, 48 h postmortem. All subcutaneous and intramuscular fat and visible connective tissues were removed from the fresh ham muscles.

The ground meat samples were produced using the following formulation: 73.5% lean pork meat, 20% pork back fat, 5% Ice, and 1.5% salt. The lean pork meat and pork back fat were ground through a 3 mm grinding plate and then the ice and salt were added. Ethanolic extracts of pumpkin leaf (CIE L\*: 9.02, CIE a\*: 0.59, and CIE b\*: 4.96) were added at a final concentration of 50% (w/w) according to the following formulation: Control (without antioxidant); PE-0.05 (with 0.05% pumpkin leaf ethanolic extract); PE-0.1 (with 0.1% pumpkin leaf ethanolic extract); PE-0.2 (with 0.2% pumpkin leaf ethanolic extract); and As-0.05 (with 0.05% ascorbic acid). These percentages were based on the formula weight of the ground meat samples without the antioxidant extract. Samples were hand mixed for 5 min. The mixed meat was then anaerobically packed in PE/nylon film bags, spread to a thickness of 2.5 cm and, stored at 4±1°C for 10 d.

### Proximate composition

The proximate properties of the samples were determined using standard AOAC (2000) methods. The mois-

ture content was determined based on the weight loss after 12 h of drying at 105°C in a drying oven (SW-90D, Sang Woo Scientific Co., Korea). The fat content was determined using the Soxhlet method with a solvent extraction system (Soxtec® Avanti 2050 Auto System, Foss Tecator AB, Sweden). The protein content was determined using the Kjeldahl method with an automatic kjeldahl nitrogen analyzer (Kjeltec® 2300 Analyzer Unit, Foss Analytical AB, Sweden) and the ash content was determined according to the AOAC (2000) method.

### pH values

The pH values of the samples were measured using a pH meter (Model 340, Mettler-Toledo GmbH, Switzerland). The pH of the raw ground pork was measured after blending 5 g of sample with 20 mL of distilled water for 60 s in a homogenizer (Ultra-Turrax SK15, Janke & Kunkel, Germany).

### Microbiological analysis

A 5 g aliquot of each sample was aseptically transferred into a sterile stomacher bag at each respective sampling interval and 45 mL of sterile distilled water was added. The sample was then evenly mixed in the stomacher (Masticator-Paddle-Blender, IUL Instrument, Spain) for 2 min at normal speed and aliquots were plated out directly at a 1:10 dilution in sterile distilled water. After serially diluting each sample in sterile distilled water, 0.1 mL were separately plated onto plates. The total bacterial count was determined on plate count agar (PCA, Difco, USA) at 35°C for 48 h. Total viable counts (TVC) were counted and expressed as Log CFU/g pork meat.

### Instrumental color measurement

The instrumental color analyses of the raw pork patties were conducted as follows. The color measurements were acquired using a colorimeter (Chroma meter CR-210, Minolta, Japan; illuminate C, calibrated with a white standard plate CIE L\* = 97.83, CIE a\* = -0.43, CIE b\* = +1.98), which consisted an 8 mm diameter measuring area and a 50 mm diameter illumination area. The color values (CIE L\*, a\*, and b\*) were measured on the sample surfaces and data were collected in triplicate for each sample.

### Thiobarbituric acid reaction substance (TBARS) values

Lipid oxidation was assessed in triplicate using the 2-thiobarbituric acid (TBA) assay described by Tarladgis *et*

*al.* (1960) with minor modifications. Fifty milliliters of distilled water was added to 10 g of sample prior to homogenizing with a homogenizer (AM-7, Nihonseiki Kaisha Ltd., Japan) at 10,000 rpm for 2 min. The cup used for blending was washed with an additional 47.5 mL of distilled water, which was added to the same distillation flask containing 2.5 mL of 4 N HCl and a few drops of an antifoam agent, silicone o/w (KMK-73, Shin-Etsu Silicone Co., Ltd., Korea). The mixture was distilled and 50 mL distillate was collected. Five mL of 0.02 M 2-thiobarbituric acid in 90% acetic acid (TBA reagent) was added to a vial containing 5 mL of the distillate and mixed well. The vials were capped and heated in a boiling water bath for 30 min to develop the chromogen and cooled to room temperature. The absorbance was measured at 538 nm, against a blank prepared with distilled water (5 mL) and TBA-reagent (5 mL), using a UV/VIS spectrophotometer (Libra S22, Biochrom Ltd., England). TBARS values were calculated by multiplying the absorbance by 73%, which was the recovery of the standard from meat, resulting in a K value of 7.8. The TBA values were calculated as mg MDA/kg sample.

$$\text{TBA (malonaldehyde mg/sample kg)} = \text{OD value} \times 7.8$$

#### Conjugated dienes (CD) and free fatty acids (FFA)

Lipid extraction was conducted according to the method described by Folch (Folch *et al.*, 1957) using a chloroform:methanol solvent system (2:1). The lipid extracts were evaporated and concentrated in a rotary evaporator (Rotary evaporator N-1000, Eyela, Japan). The extracted lipids were placed then analyzed by CD and FFA. The CD concentrations were determined as described by Prasetyo *et al.* (2008). Fifteen mg of extracted lipid sample was placed into a 25 mL volumetric flask and massed up with isooctane. The samples were mixed and the absorbance was read at 234 nm against a blank of isooctane using a UV/VIS spectrophotometer (Libra S22, Biochrom Ltd., England). The CD concentration was calculated using a molar extinction coefficient of 25,200 M<sup>-1</sup> cm<sup>-1</sup> and expressed as  $\mu\text{mol mg}^{-1}$  meat lipid sample.

The free fatty acids (FFA) values of the extracted lipids were determined by AOCS (1987), and calculated as follows:  $\text{FFA (\%)} = (S - B) \times M_0 \times N \times F/W$  [S = titration amount of sample; B = titration amount of blank; M<sub>0</sub> = molecular weight of KOH; F = titer of 0.01 N KOH; N = normality of KOH; W = sample weight (g)].

#### Statistical analysis

Analysis of variance was performed on all the variables measured using the General Linear Model (GLM) procedure of the SAS statistical package (SAS Institute, Inc., 2008). Duncan's multiple range test ( $p < 0.05$ ) was used to determine the differences between treatment means.

## Results and Discussion

### Proximate composition

The addition of PE did not have an effect on the proximate composition of the ground pork (Table 1). The moisture content of ground pork samples ranged from 63.35-63.78%. No significant differences were observed in moisture, protein, fat and ash content among the treatments.

### pH evaluations

The pH values of the ground pork samples containing ethanolic pumpkin leaf extract ranged from 5.87 to 6.00 after 10 d of storage (Table 2). In addition, the pH increased ( $p < 0.05$ ) with an increase in the pumpkin leaf extract concentration. The pH of all treatments decreased up to 7 d of storage, except for PE1. After 7 d, the pH increased ( $p < 0.05$ ). A similar study reported that pH values of ground pork containing garlic extracts decreased during the first 4 d of chilled storage, and significantly increased ( $p < 0.05$ ) thereafter (Byun *et al.*, 2001). Also, pH changes were observed in pork patties containing aloe vera, fenugreek, mustard, and rosemary extracts (McCarthy *et al.*, 2001).

### Total viable counts (TVC)

The TVC ranged from 2.45 Log CFU/g to 4.65 Log CFU/g for the ground pork samples containing PE during

**Table 1. Proximate composition of ground pork meat added ethanolic pumpkin leaf extracts (PE)**

Treatment <sup>1)</sup>	Proximate composition (%)			
	Moisture	Protein	Fat	Ash
Control	63.51±0.24	15.90±0.36	17.49±0.35	2.06±0.07
PE-0.05	63.64±0.15	16.37±0.23	17.42±0.33	2.13±0.10
PE-0.1	63.43±0.23	15.90±0.35	17.73±0.51	2.24±0.15
PE-0.2	63.78±0.40	16.51±0.40	17.26±0.33	2.07±0.08
As-0.05	63.35±0.14	16.36±0.27	17.69±0.28	1.95±0.14

All values are mean±SD of three replicates.

<sup>1)</sup>Control, minced pork without antioxidant powder; PE-0.05, minced pork meat with 0.05% pumpkin leaf extracts; PE-0.1, minced pork meat with 0.1% pumpkin leaf extracts; PE-0.2, minced pork meat with 0.2% pumpkin leaf extracts; As-0.05, minced pork meat with 0.05% ascorbic acid

**Table 2. Change in pH values of ground pork meat added ethanolic pumpkin leaf extracts (PE) during storage days**

Treatment <sup>1)</sup>	Storage periods (d)			
	1	4	7	10
Control	5.87±0.09 <sup>Cb</sup>	5.90±0.05 <sup>Ba</sup>	5.93±0.06 <sup>Aa</sup>	5.87±0.09 <sup>Cb</sup>
PE-0.05	5.89±0.09 <sup>C</sup>	5.91±0.10 <sup>B</sup>	5.88±0.07 <sup>C</sup>	5.88±0.05 <sup>BC</sup>
PE-0.1	5.88±0.06 <sup>Cb</sup>	5.92±0.07 <sup>Ba</sup>	5.88±0.07 <sup>Cb</sup>	5.90±0.03 <sup>Bab</sup>
PE-0.2	5.94±0.08 <sup>Ba</sup>	5.93±0.04 <sup>ABa</sup>	5.91±0.08 <sup>Bb</sup>	5.92±0.05 <sup>Bab</sup>
As-0.05	6.00±0.06 <sup>Aa</sup>	5.96±0.09 <sup>Ab</sup>	5.93±0.03 <sup>Ac</sup>	5.96±0.08 <sup>Ab</sup>

All values are mean±SD of three replicates.

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

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

<sup>1)</sup>Control, minced pork without antioxidant powder; PE-0.05, minced pork meat with 0.05% pumpkin leaf extracts; PE-0.1, minced pork meat with 0.1% pumpkin leaf extracts; PE-0.2, minced pork meat with 0.2% pumpkin leaf extracts; As-0.05, minced pork meat with 0.05% ascorbic acid

10 d of storage (Table 3). Generally, total viable counts above 7 Log CFU/g can result in visible meat spoilage during aerobically refrigerated storage (Insausti *et al.*, 2001). The TVC of the control increased to about 2 Log CFU/g over the 10 d of storage. The TVC increased, between approximately 1.3 and 1.5 Log CFU/g, up to day 10 in the samples containing PE. The TVC was inhibited most by the PE-0.2 treatment (0.8 Log CFU/g). Although PE was added to the pork samples, TVC inhibition may not have been affected by pH, which clearly did not increase in the samples containing PE during the storage

**Table 3. Total aerobic bacterial counts (log<sub>10</sub> CFU/g) of ground pork meat added ethanolic pumpkin leaf extracts (PE) during storage days**

Treatment <sup>1)</sup>	Storage periods (d)			
	1	4	7	10
Control	2.76±0.05 <sup>Ad</sup>	3.11±0.03 <sup>Ac</sup>	3.95±0.04 <sup>Ab</sup>	4.65±0.05 <sup>Aa</sup>
PE-0.05	2.58±0.03 <sup>Bd</sup>	2.83±0.04 <sup>Dc</sup>	3.65±0.06 <sup>Cb</sup>	4.02±0.01 <sup>Ca</sup>
PE-0.1	2.45±0.02 <sup>Dd</sup>	2.94±0.01 <sup>Cc</sup>	3.49±0.04 <sup>Db</sup>	3.94±0.03 <sup>Da</sup>
PE-0.2	2.51±0.03 <sup>Cd</sup>	2.67±0.06 <sup>Ec</sup>	3.38±0.03 <sup>Eb</sup>	3.87±0.02 <sup>Ea</sup>
As-0.05	2.52±0.03 <sup>Cd</sup>	2.99±0.01 <sup>Bc</sup>	3.75±0.06 <sup>Bb</sup>	4.35±0.10 <sup>Ba</sup>

All values are mean±SD of three replicates.

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

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

<sup>1)</sup>Control, minced pork without antioxidant powder; PE-0.05, minced pork meat with 0.05% pumpkin leaf extracts; PE-0.1, minced pork meat with 0.1% pumpkin leaf extracts; PE-0.2, minced pork meat with 0.2% pumpkin leaf extracts; As-0.05, minced pork meat with 0.05% ascorbic acid

time. Decreases in TVC appeared to be an effect of PE.

### Instrumental color evaluation

Discoloration in meat is interrelated with lipid oxidation and metmyoglobin production through the action of free radicals (O'Grady *et al.*, 2001). The redness of meat is important to consumer acceptability. In all samples, CIE L\* values significantly increased ( $p<0.05$ ) and CIE a\* values decreased ( $p<0.05$ ) over 10 d of storage (Table 4). Increases in the PE concentration resulted in darker raw ground pork relative to the control during the entire storage period. The ground pork samples with PE had lower CIE a\* values than the control. However, higher CIE a\* values were observed on day 4 for PE-0.1, PE-0.2, and As-0.05 samples as compared to day 1. Also, changes in redness were lowest in PE-0.1, and similar changes in values were observed for As-0.05, which can prevent discoloration with increasing storage time. Similar effects were observed in raw patties containing green tea ethanolic extract (Jo *et al.*, 2003). The changes in redness were lower for samples containing PE, which this result indicates that PE is an effective agent for color stability. The addition of PE resulted in increased CIE b\* values during the entire storage period, which may have been due to the deep green color of the PE.

### Conjugated dienes (CD) and Free fatty acids (FFA)

Lipid oxidation in meat can result in quality deterioration and decreases in sensory and nutritional factors (Juntachote *et al.*, 2007). Unsaturated lipids that have non-conjugated double bonds transform into conjugated dienes after peroxides are formed during lipid oxidation. Hydroperoxides hardly decompose during the early stage of lipid oxidation and decompose into secondary products at the later stage (Kulas and Ackman, 2001). Lipid oxidation can be measured based on the CD concentrations. The effects of PE on CD during storage are presented in Fig. 1. In the control, CD significantly increased until day 4, and then significantly decreased until day 10. Samples containing PE had increased concentrations of CD during storage from day 1 to day 7, which concentrations significantly decreased at longer storage times ( $p<0.05$ ). This result may be due to decomposition into secondary products of conjugated diene hydroperoxides after peaked CD formation (Juntachote *et al.*, 2007). Peña-Ramos and Xiong (2003) found that conjugated dienes in cooked pork patties made with hydrolyzed protein isolate (WPI) and soy protein isolate (SPI) increased until day 1, and then decreased after day 1, except for the control. The

**Table 4. Change in color stability of ground pork meat added ethanolic pumpkin leaf extracts (PE) during storage days**

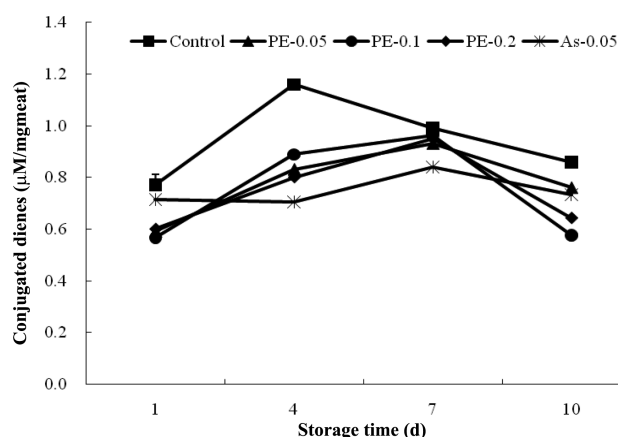
	Treatment <sup>1)</sup>	Storage periods (d)			
		1	4	7	10
CIE L*	Control	57.44±0.70 <sup>Cb</sup>	55.65±0.97 <sup>Cc</sup>	58.73±0.57 <sup>Bb</sup>	61.76±0.93 <sup>Aa</sup>
	PE-0.05	60.74±0.96 <sup>Aa</sup>	61.06±1.08 <sup>Ba</sup>	57.81±1.35 <sup>Bb</sup>	60.95±1.05 <sup>Ba</sup>
	PE-0.1	60.50±0.66 <sup>ABa</sup>	56.25±0.73 <sup>Cb</sup>	52.71±0.59 <sup>Cc</sup>	60.05±0.89 <sup>Ca</sup>
	PE-0.2	55.97±0.64 <sup>Db</sup>	56.86±1.03 <sup>Cb</sup>	51.90±1.30 <sup>Dc</sup>	60.03±1.28 <sup>Ca</sup>
	As-0.05	59.65±0.61 <sup>Bd</sup>	63.67±1.16 <sup>Aa</sup>	61.36±0.80 <sup>Ac</sup>	61.24±0.96 <sup>ABbc</sup>
CIE a*	Control	18.38±0.79 <sup>Aa</sup>	17.44±0.79 <sup>ABb</sup>	11.92±0.99 <sup>Bc</sup>	9.10±0.41 <sup>Cd</sup>
	PE-0.05	17.19±0.92 <sup>Ba</sup>	14.97±0.99 <sup>Cb</sup>	11.69±0.96 <sup>Bc</sup>	8.89±0.79 <sup>Cd</sup>
	PE-0.1	14.96±0.59 <sup>Cb</sup>	18.02±0.72 <sup>Aa</sup>	12.55±0.91 <sup>Bc</sup>	9.05±0.96 <sup>Cd</sup>
	PE-0.2	13.03±1.16 <sup>Db</sup>	16.74±0.88 <sup>Ba</sup>	10.63±0.74 <sup>Cc</sup>	6.14±0.57 <sup>Dd</sup>
	As-0.05	15.59±0.94 <sup>Cb</sup>	16.68±0.98 <sup>Ba</sup>	15.16±0.97 <sup>Ab</sup>	11.13±0.90 <sup>Ac</sup>
CIE b*	Control	15.29±0.95 <sup>Ba</sup>	14.51±0.83 <sup>Ca</sup>	11.96±0.86 <sup>Db</sup>	15.15±0.75 <sup>Ba</sup>
	PE-0.05	16.18±0.94 <sup>Aa</sup>	16.61±1.12 <sup>Ba</sup>	13.15±0.99 <sup>Cc</sup>	15.02±1.47 <sup>Bb</sup>
	PE-0.1	15.76±0.84 <sup>ABb</sup>	18.87±0.95 <sup>Aa</sup>	13.84±0.41 <sup>BCc</sup>	15.22±0.88 <sup>Bb</sup>
	PE-0.2	15.59±0.94 <sup>ABc</sup>	19.67±0.81 <sup>Aa</sup>	15.98±0.83 <sup>Abc</sup>	16.81±0.97 <sup>Ab</sup>
	As-0.05	14.20±0.69 <sup>Cb</sup>	17.43±0.94 <sup>Ba</sup>	14.39±0.73 <sup>Bb</sup>	12.86±0.96 <sup>Cc</sup>

All values are mean±SD of three replicates.

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

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

<sup>1)</sup> Control, minced pork without antioxidant powder; PE-0.05, minced pork meat with 0.05% pumpkin leaf extracts; PE-0.1, minced pork meat with 0.1% pumpkin leaf extracts; PE-0.2, minced pork meat with 0.2% pumpkin leaf extracts; As-0.05, minced pork meat with 0.05% ascorbic acid



**Fig. 1. Change in conjugated dienes ( $\mu\text{M}/\text{mg}$  meat) of ground pork meat added ethanolic pumpkin leaf extracts (PE) during storage days.**

addition of PE decreased the CD concentrations to levels lower than the control during the entire storage period. In particular, PE-0.1 and PE-0.2 had the lowest ( $p<0.05$ ) CD concentration on day 10. Seo and Morr (1984) reported that antioxidant compounds like phenolics may lower CD concentrations. Furthermore, a previous study reported that pumpkin leaves have antioxidant activity (Cha, 2009).

Decomposition values of tri-glycerides and phospholipids, which are present in meat during storage, can be

measured through free fatty acid (FFA) analysis (Dempster *et al.*, 1985). The addition of PE delayed the formation of FFAs in ground pork during chilled storage, which ranged in concentration from 0.56 to 2.23 % (Table 5). The samples containing PE had significantly lower ( $p<0.05$ ) FFA content compared to the control and AS-0.05 treatment. Also, PE concentration had a significant ( $p<0.05$ ) effect on FFA content. Increases in FFA content were dependent on storage time for all treatments. A previous study found that the FFA percentage increased with increasing storage time (Lefebvre *et al.*, 1994).

### Thiobarbituric acid reaction substances (TBARS) values

Fig. 2 showed the TBARS values, which can be used as a measure of the concentrations of secondary lipid oxidation products such as aldehydes or ketones in samples containing PE (0.18-1.05 MDA mg/kg meat). Previously, a study found slightly higher TBARS levels (0.84-2.05 MDA mg/kg), in raw chicken patties during 13 d of storage at 4°C. The TBARS values of the samples significantly increased ( $p<0.05$ ) with increasing storage time. Similar results were also observed in fresh minced beef, where the TBARS values increased with storage (up to 5 mg MDA/kg sample) (Tang *et al.*, 2006). The control in particular had the highest TBARS values during the

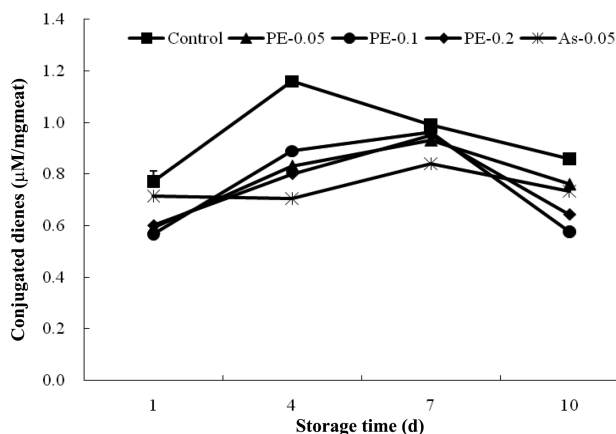


Fig. 1. Change in conjugated dienes ( $\mu\text{M}/\text{mg}$  meat) of ground pork meat added ethanolic pumpkin leaf extracts (PE) during storage days.

entire storage period. In the PE samples, the increase in TBARS values was slow and was lower ( $p < 0.05$ ) level than in the Control. This result indicates that PE inhibited lipid oxidation in the meat samples due to the presence of phenolic compounds and antioxidant vitamins. Liu *et al.* (2009) reported decreased TBARS values in chicken sausage containing rosemary and Chinese mahogany relative to the control. Decreases in CDs concentration caused an increase in the production of secondary products such as TBARS during storage (Juntachote *et al.*, 2007). The TBARS values of the 0.2% PE sample remained relatively constant during storage relative to As-0.05 (Morris *et al.*, 1950). This was most likely observed because this treatment contained the highest concentration of PE, and thus the highest amount of phenolic compounds. The TBARS value increased the most for the control and As-0.05 samples due to the re-decomposition of peroxide between storage day 7 to 10. Byun *et al.* (2001) also

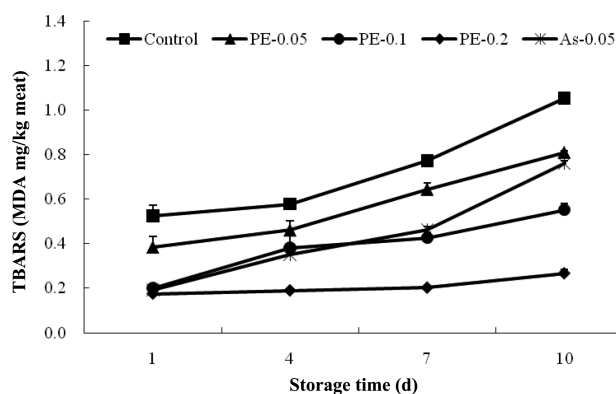


Fig. 2. Change in thiobarbituric acid reaction substance (TBARS) values (mg of MDA/kg meat) of ground pork meat added ethanolic pumpkin leaf extracts (PE) during storage days.

Table 5. Change in free fatty acid (FFA) (%) of ground pork meat added ethanolic pumpkin leaf extracts (PE) during storage days

Treatment <sup>1)</sup>	Storage periods (d)			
	1	4	7	10
Control	1.23±0.32 <sup>A</sup> <sub>c</sub>	1.54±0.24 <sup>A</sup> <sub>b</sub>	1.75±0.08 <sup>A</sup> <sub>b</sub>	2.23±0.14 <sup>A</sup> <sub>a</sub>
PE-0.05	0.78±0.26 <sup>C</sup> <sub>b</sub>	0.83±0.22 <sup>C</sup> <sub>b</sub>	1.18±0.03 <sup>C</sup> <sub>a</sub>	1.44±0.18 <sup>C</sup> <sub>b</sub>
PE-0.1	0.62±0.12 <sup>D</sup> <sub>c</sub>	0.84±0.16 <sup>C</sup> <sub>b</sub>	1.12±0.25 <sup>C</sup> <sub>b</sub>	1.25±0.06 <sup>D</sup> <sub>a</sub>
PE-0.2	0.56±0.11 <sup>D</sup> <sub>c</sub>	0.61±0.04 <sup>D</sup> <sub>c</sub>	0.84±0.03 <sup>D</sup> <sub>b</sub>	0.96±0.52 <sup>E</sup> <sub>a</sub>
As-0.05	1.00±0.05 <sup>B</sup> <sub>c</sub>	1.13±0.03 <sup>B</sup> <sub>c</sub>	1.38±0.17 <sup>B</sup> <sub>b</sub>	1.52±0.32 <sup>B</sup> <sub>a</sub>

All values are mean±SD of three replicates.

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

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

<sup>1)</sup> Control, minced pork without antioxidant powder; PE-0.05, minced pork meat with 0.05% pumpkin leaf extracts; PE-0.1, minced pork meat with 0.1% pumpkin leaf extracts; PE-0.2, minced pork meat with 0.2% pumpkin leaf extracts; As-0.05, minced pork meat with 0.05% ascorbic acid

observed sharp increases in TBARS values in pork after 8 d of storage.

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