Evaluation of antioxidant potential of *Cudrania tricuspidata* (CT) Leaves, Fruit powder and CT Fruit in pork patties During storage

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Abstract

The objective of this study was to assess antioxidant activities of leaves and fruit powder of *Cudrania tricuspidata* (CT) with different particle sizes (crude, 500 μm, 150 μm), physicochemical properties and the effect of various levels of CT fruit powder (CTFP) on the product quality of pork patties. Total phenolic content of crude leaves had the highest value of 3.54 g/100 g ($p<0.05$). Overall, CT leaves (CTLP) had higher total phenolic content than CTFP ($p<0.05$). DPPH radical scavenging activity of CTFP was higher than that of CTLP ($p<0.05$). CTLP showed higher iron chelating ability and reducing power than CTFP ($p<0.05$). After pork patties were manufactured with 0.5 and 1.0% of CTFP at 500 μm, pH, color values, thiobarbituric acid reactive substance (TBARS), and peroxide values (POV) were then measured. The addition of CTFP into pork patties significantly ($p<0.05$) increased redness and yellowness values of patties. TBARS values of pork patties containing CTFP were lower ($p<0.05$) than those of CTFP-0 patties after 10 days of storage. Pork patties added with CTFP showed no significant ($p>0.05$) difference TBARS values among different storage periods. POV values of pork patties containing CTFP were lower than those of the control from 3 days up to the end of refrigerated storage ($p<0.05$). These results suggest that CTFP could be used as a natural antioxidant to retard lipid oxidation in meat products during refrigerated storage.

Keywords: *Cudrania tricuspidata*, antioxidant, lipid oxidation, pork patties

1. Introduction

Since consumers tend to select high quality of food rather than a high quantity of food with increased economic development, the development of processed meat with natural ingredients has been focused on functional meat products. (Cardenia et al., 2011; Choe et al., 2013). These functional properties include antioxidant and antimicrobial activities as well as other bioactive actions such as reducing blood pressure and increasing immunity. It is known that lipid oxidation of meat products caused by autoxidation and hydrolysis deteriorates the quality of food. The autocatalytic chain reaction of active oxygen and free radicals can produce peroxides such as wide range of carbonyl compounds, furans, hydrocarbons (Kanner, 1994). These secondary oxidative products have undesirable effects on the quality and nutritional value of products such as odor, texture, color, and flavor (Fernández et al., 1997).

Therefore, antioxidants should be used to delay lipid oxidation in many foods since antioxidants can interfere with the formation of free fatty acids and thus inhibit oxidation reactions (Kelleher et al., 1992). Many synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) have been used as antioxidants. However, they might have been found that these synthetic antioxidants induce toxicity and carcinogenic (Branen, 1975, Hirose et al., 1998). In particular, Festjens et al. (2006) reported that BHA shifts cell death from necrosis to apoptosis. Thus, application and research of natural antioxidants to meat products have been actively carried out (Biswas et al., 2012; García-Lomillo et al., 2017).

The color of meat or meat products is a specific visual appearance characteristic that affects consumer choice at the point of purchase of the meat (Ngapo et al., 2007). Consumers prefer pink in cured meat products, so color formation is one of the important sensory properties of meat products that affect consumption (Zhang et al., 2007). To these consumers' preferences, colorants are added to meat products, but due to allergenic and intolerance reations to synthetic colorants, consumers have increased interest in natural colorant, which is associated with a healthy image (Ø sterlie and Lerfall, 2005).

Cudrania tricuspidata Bureau (CT) is a deciduous arboreous of the moraceae tree with blooms in May-June. The color of this fruit is red or purple. Its leaves have been used as food for silkworms, while its berries are used for food and medicinal purpose (Sol, 2006). Many studies have been performed on CT's physiological activities such as cytotoxic (Kim et al., 2012), antibacterial (Choi et al., 2009), and antiallergic (Oh et al., 2009) activities. CT leaves extracted with ethanol and methanol were applied to

pork meat patties to extend shelf life (Cuong and Chin, 2018). However, there was not much study on the antioxidant activity as affected by particle size of the CT leaves and fruit powder. The direct incorporation of natural powder from CT fruit to processed meat products may be an effective and economic strategy to control meat products' oxidation. Furthermore, it is expected that the red color of meat products will be enhanced due to the color of CT fruits. In this study, the antioxidant activities by TBARS, POV assay, physicochemical, and microbiological of pork patties, added with various concentrations of CT fruit powder during storage, were evaluated.

2. Materials and Methods

Experiment I. Antioxidant activity of CT leaves and fruits

2.1. Materials

CT fruits and leaves harvested in September 2018 were purchased at local commercial markets (Hampyeong, Jeollanam-do, Korea). After removing discolored and damaged parts, the samples were washed with water before being dried. Leaves were dried with hot air in an oven at 60℃ and fruits were dried using a freeze dryer under a pressure of 7 mm Torr at –50℃. These dried leaves and fruits were ground using a grinder (HMF Electric -3260s, Seoul, Korea). Then some of the homogenized CT leaves and fruits were kept unfiltered in polyethylene bags (CTLP, CTFP). After the rest were sieved with the size of 500 μm mesh sieve (ChungGye Industrial Mfg., Co., Korea), some of the sieved powder was stored in a polyethylene bag (CTLP 500 μm, CTFP 500 μm). Another part of the sieved powder was sieved again to a size of 150 mesh sieve, and then the filtered powder was stored in a polyethylene bag (CTLP 150 μm, CTFP 150 μm). All powders were stored at -70 ℃ and used in the experiment.1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteau reagent, ethylenediaminetetraacetic acid (EDTA), 2-thiobarbituric acid (TBA) and ammonium thiocyanate were all purchased from Sigma-Aldrich (Steinheim, Germany). L(+) Ascorbic acid, ferrous chloride, ferric chloride, gallic acid, trichloroacetic acid, petroleum ether and barium chloride anhydrous were purchased from Junsei Chemical (Tokyo, Japan). Ferrous sulfate heptahydrate was supplied by Duksan Pure Chemicals (Kyungkido, Korea). Potassium ferricyanide was purchased from Avocado Reserch Chemicals (Lancaster, UK). Methanol

(99.5% purity) was obtained from OCI company Ltd.(Seoul, Korea).

2.2. Total phenolic content

Total phenolic content was determined using the method described by Lin and Tang (2007). The sample solution was prepared at a concentration of 10 mg/mL. Approximately 2 mL of 2% (w/v) sodium carbonate was added to 0.1 mL of each sample in a test tube. Then, 1 mL of Folin-Ciocalteau reagent (previously diluted 2-fold with distilled water) were added and shaken. The mixture was allowed to stand at room temperature for 30 min. Absorbance was measured at a wavelength of 750 nm. Contents of phenolic compounds were then calculated using a standard curve of gallic acid at 0 - 200 mg/L concentrations and expressed as gallic acid equivalents / 100 g of dried powder weight.

2.3. DPPH (1,1-Diphenyl-2-picryl hydrazyl)-radical scavenging

DPPH radical scavenging activity was determined with the method of Huang et al. (2006). Briefly, 0.5 mL of DPPH (0.2 mM in methanol) was added to 2 mL of extract solution at different concentrations diluted with dd-water (0, 1, 2.5, 5.0, and 10 mg/mL). The mixture was left to stand for 30 min in darkness. Its absorbance was measured at a wavelength of 517 nm against a blank. Ascorbic acid was used as the reference (REF). DPPH-radical scavenging activity was calculated with the following equation: DPPH radical scavenging activity (%) = $(1-A/B) \times 100$, where A was the absorbance of the sample and B was the absorbance of the control.

2.4. Iron chelating ability

Iron chelating ability was determined using the method described by Le et al. (2007). After preparing CT leave and fruit powder samples at different concentrations diluted with dd-water (0, 1, 2.5, 5.0, and 10 mg/mL), 0.5 mL of each sample, 0.1 mL of $FeCl_2$ (0.6 mM in water), and 0.9 mL of methanol were mixed and reacted at room temperature for 5 min. After 0.1 mL of ferrozine (5 mM) was added, the mixture was kept at room temperature for 10 min. Absorbance was measured at 562 nm. EDTA was used as a positive control. Iron chelating ability was calculated with the following equation: Iron

chelating ability (%) = (1−A/B) × 100, where A was the absorbance of the sample and B was the absorbance of the control.

2.5. Reducing Power

Reducing power was determined using the method described by Huang et al. (2006). Briefly, 2.5 mL of $K_3Fe(CN)_6$ (1 % (w/v)) and 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) were added to each sample (0, 1, 2.5, 5.0, and 10 mg/mL) dissolved in distilled water. Reaction mixtures were mixed well and incubated at 50℃ in an oven (LDO-250F, Labtech co., LTD., Korea) for 20 min. After 2.5 mL of 10% (w/v) trichloroacetic acid was added, mixtures were centrifuged at 1476×g for 10 min. From the upper layer, 2.5 mL solution was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1% (w/v)). The mixture was then incubated at room temperature for another 10 min. Absorbance was then measured spectrophotometrically at 700 nm. Ascorbic acid was used as a reference treatment.

2.6. High-performance liquid chromatography (HPLC) analysis of individual antioxidant components

The individual antioxidant components were determined by the method of Kwon et al. (2016) with slight modificaions. A 9 g of CTLP and CTFP were weighed each, extracted with 90 mL of 80% methanol on a magnetic stirrer for 24 h at room temperature and centrifuged (VS-5000N, Vision scientific Co. Ltd., Korea) at 2214×g for 10 min to collect the supernatant. This operation was repeated 3 times. The supernatants were pooled an condensed using a rotary evaporator (R-100 Rotary Evaporator, Switzerland) to remove methanol at 40℃. Then, the concentrate was dissolved in 5 mL with methanol. All samples were passed through a 0.5 μm filtermembrane for HPLC analysis Analytic HPLC was conducted on a LC-20AVP HPLC system (shimadzu, Japan) fitted with a C18 reverse- phase (5 μm) column (250 × 4.6 mm). A mixture of water/acetonitrile, 90:10 (A), and 10: 90 (B) was used as a mobile phase. The 1.0 mL/ min flow rate was kept constant during the measurement and an injection volume of 20 μL in all experiments. The gradient elution was performed as following protocol: 0-2 min, 100% A, 6-31 min 10-63% B, and 41-45 min 50-100% A. The compounds were tested at the same

154 wavelengths (nm) 280 for gallic acid, ferulic acid, chlorogenic acid, rutin, and isovanillic acid standards.
155 These compounds were eluted from the column with retention time of 4.2, 10.8, 13.4, 15.3 and 17.0
156 respectively. The quantification of the phenolic and flavonoid compounds from CTLP and CTFP was
157 performed by comparing standard spectra at each retention time

158 **Experiment II: Product quality of pork patties with CT fruits powder**

159 **2.7. Manufacture of Pork Patties**

160 Pork ham (*Semimembranosus*) used in the manufacture of pork patties added with CT fruit powder
161 (CTFP) was purchased from a local commercial meat processing company (Samho, Gwangju, Korea).
162 After removing connective tissue and fat, hams were ground through a grinder (Meat chopper, M-12S,
163 Hankook Fujee Industries, Korea) using grinder plates of 5 mm hole diameter. After additives were
164 added and mixed thoroughly with a mixer (EF20, Crypto Peerless Ltd, Birmingham, UK), the sample
165 was re-homogenized using a grinder with 8 mm hole diameter grinder plate, divided into 65-70 g, and
166 homogenized again. The sample was then shaped using a petri dish. The formulation of pork patties
167 added with CTFP is shown in Table 1. Patties were made without (CTFP-0) or with 0.1 g/ 100 g of
168 ascorbic acid (REF), 0.5 g/ 100 g of freeze-dried CTFP with size below 500 μm (CTFP-0.5), and 1.0 g/
169 100 g of freeze-dried CTFP with size below 500 μm (CTFP-1.0). These prepared pork patties were stored
170 in a polystyrene box at 4 ± 1℃ for 14 days. The different measurement was done initially and at days
171 3, 7, 10, and 14 of the storage. Patties were analyzed for color, pH, thiobarbituric acid reactive
172 substances (TBARS), and peroxide values (POV). All experiments were repeated three times.

173

174 **2.8. pH and color**

175 The color of each pork patty sample was measured using a color meter (CR-10, Minolta Co, LTD,
176 Japan) using an 8 mm measuring aperture size, with a CIE standard illuminant D_{65} and 10˚ observer.

177 The patty surface was repeatedly measured six times and the average value was calculated. Lightness
178 (L *, lightness), redness (a *, redness), and yellowness (b *, yellowness) values were shown. The pH
179 of pork patty sample was measured using a pH-meter (Model 120, Mettler-Toledo, Switzerland). The
180 pH value of this experiment was obtained as an average value after repeating the measurement five

times.

2.9. TBARS

Levels of TBARS were determined using the method, according to Shinnhuber and Yu (1977). TBARS indicates fatty acid level by measuring O.D. values after adding TBA reagent, which reacts with malondialdehyde (MDA) and expresses red color. Briefly, 2 g of homogenized meat sample was blended with 3 mL of 1% (w/v) thiobarbituric acid and 17 mL of 2.5% (w/v) trichloroacetic acid in glass tubes. The blank sample consisted of 17 ml of 2.5% (w/v) trichloroacetic acid, 3 ml distilled water. The mixture was then heated at 100℃ for 30 min. After mixing 5 mL of chloroform and 5 mL of the reacted sample in a conical glass tube by vortexing, the mixture was centrifuged at 1476×g for 5 minutes. Then 3 mL of the supernatant from each a conical glass tube and 3 mL of petroleum ether were mixed and centrifuged again at 1476×g for 10 min. The absorbance of the supernatant was then read at a wavelength of 532 nm. TBARS values were calculated using the following equation:

TBARS value (mg of MDA/kg of sample) = (O.D.value × 9.48)/ meat sample weight (g)

9.48 was a constant derived from the sample dilution factor and the absorption coefficient (152,000 M^{-1} cm^{-1}) of the red thiobarbituric acid reaction product.

2.10. POV

POV was determined according to the method of Shantha and Decker (1994). Briefly, 0.3 g of pork patty sample was homogenized with a mixture of 5 mL of chloroform and methanol (ratio of 1:1 v/v). After mixing 3.08 mL of 5 (mg/ ml) sodium chloride and the homogenized sample in a tube, the mixture was vortexed for 30 seconds and centrifuged at 1476×g for 5 min. After centrifugation, 2 mL of the supernatant was added to the bottom of a tube and mixed with 1.33 mL of chloroform and methanol (1:1, v/v). Then 25 μL of ammonium thiocyanate reagent (30 g / 100 mL) and iron (II) chloride were added to the sample, respectively. The mixture was mixed for 5 seconds and incubated at room temperature for 20 min. The absorbance of the mixture was then measured at a wavelength of 500 nm against a blank that contained all the reagents except the sample. A standard calibration curve was used

with a concentration range of 0.1 – 1.0 (μg/ mL). POV was determined as milliequivalents per kilogram of fat.

2.11. Microbiological analyses

Total plate count (TPC) agar (Difco, USA) and violet red bile (VRB) agar (Difco, USA) were prepared for the determination of pork patties containing CT fruit of total bacterial counts and *Enterobacteriaceae*. A 10 g of each sample was transferred to sterile vacuum package pouches containing 90 mL of water. Then, the pork patties in the pouch were homogenized for 20 s using a laboratory stomacher Lab-Blender (Bagmixer 400, Interscience., France). Dilutions (10^{-1}, 10^{-2}, and 10^{-3}) were prepared and spread onto TPC and VRB agar plates. Then, plates were incubated at 37 ± 1℃ for 48 h. Then, the number of colonies was counted. The result was represented by using $\log_{10}$ colony-forming units per gram (CFU/g).

2.12. Statistical analysis

All experiments were repeated three times. All statistical analyses were performed using the SPSS 22.0 software program (SPSS Inc., Chicago, IL, USA). For antioxidant activities of CT leaves and fruit, the data were analyzed using 2-way ANOVA as factors for treatments (reference, CTLP, CTFP, CTLP 150 μm, CTFP 150 μm, CTLP 500 μm, CTFP 500 μm) and concentration (0, 1, 2.5, 5.0, and 10 mg/mL). For pork patties added with CT fruit, the data were determined using two-way ANOVA by treatments (CTFP-0, REF, CTFP-0.5, CTFP-1.0) and storage time. Duncan's multiple range test was carried out to calculate the significant difference between the means. Differences of $p < 0.05$ were considered to be significant.

3. Results and discussion

Experiment I. Antioxidant activity of CT leaves and fruits

3.1. Total phenolic content

Total phenolic content and antioxidant activities of oven-dried CTLP and freeze-dried CTFP were

analyzed according to particle size (Table 2). Content of total phenolic of all concentrations quantified ranged from 1.49 to 3.54 (g/100 g dry weight). CTLP was higher ($p<0.05$) than those in fruits regardless of particle size. Crude CTLP showed the highest total phenolic content among all samples ($p<0.05$). In the total phenolic content result of the CTFP, there was no difference in the according to the particle size ($p<0.05$). The smaller the particle size of plants with antioxidant properties, the larger the surface area, so the antioxidant capacity is well transferred to the solvent (Castiglioni et al., 2015). However, as the surface area of substances increases, O_2 can affect the stability of the antioxidant material (Hu et al., 2012). Choi et al. (2009) have determined total phenolic content in methanol extracts of various parts of CT tree and observed higher content of total phenolic in leaves compared to fruit. Hong and Hong (2015) have also reported that the fruit of CT has lower levels of total phenolic than leaves in either ethanol or water extract. Memon et al. (2010) have also reported that levels of total phenolic compounds in fruits are lower than those in leaves regardless of mulberry species. They related this result to the amount of antioxidants such as ferulic acid are present in leaves more than in fruits. However, in this study, the CTFP contained more ferulic aicd than the CTLP, whereas the CTLP had more rutin content than the CTFP. (Table 3).

3.2. DPPH radical scavenging

In this study, we confirm that the difference of antioxidative activities of the CT leaves and fruit, which is dried by the oven-drying method and freeze-drying method, respectively. As shown in Figure 1A, DPPH radical scavenging activity of CTFP was higher than that of CTLP. Among samples of fruits with different particle sizes, those with a particle size of less than 150 μm (CTFP 150 μm) showed the highest radical scavenging activity, among the treatments ($p<0.05$). There was no significant ($p>0.05$) difference in DPPH radical scavenging activity between CTLP at a particle size of 500 μm or less (CTLP 500 μm) and CTLP at a particle size of 150 μm or less (CTLP 150 μm). However, the leaves powder sample with crude particle size had lower ($p<0.05$) DPPH radical scavenging ability than leaves powder sample with particle size less than 500 μm. Chon et al. (2009) have reported that DPPH radical scavenging activity of leaves powder sample was higher than those of fruit powder samples. These results present that even if the fruit has lower total phenolic content than leaves, DPPH radical

scavenging activity might be higher. Csepregi et al. (2016) explained that different types of phenols might lead to different consequences due to different structure-activity relationships between polyphenol substituents and antioxidant activity results. Therefore, it seems that the difference in the types of phenol contained in leaves and fruit influenced the result of DPPH radical scavenging activity in this study (Natić et al., 2015).

3.3. Iron chelating ability

Iron chelating ability is one of the methods to confirm the antioxidant capacity. It is known that Fe (II) possesses the ability to speeds up the generation of hydroxyl radical through the Fenton reaction (Rastogi et al, 2009). Ferrozine combines with $Fecl_2$ to form a complex, resulting in purple color. Chelating agents may serve interrupted complex formation, resulting in a reduction in the purple color of the complex. Measurement of absorbance of the purple color reduction, therefore, allows estimation of the iron chelating activity (Gülçin et al, 2011). In the present study, the chelating ability of the CT leaves and fruits powder toward ferrous ions was investigated. Results of iron chelating ability are shown in Figure 1B. CTLP had higher ($p<0.05$) iron chelating ability than CTFP. All leave samples showed no significant ($p>0.05$) difference in iron chelating activity from EDTA at concentrations of higher than 0.1% (w/v). It has been reported that total phenolic and flavonoids contents in CTLP are higher than those in CT fruits (Choi et al., 2009). These contents can affect the iron chelating ability (Natić et al., 2015). At a concentration of 1.0% (w/v), all fruit powder samples showed more than 80 % iron chelation ability. The result of iron chelation activity according to particle size showed no difference ($p>0.05$) inactivity regardless of CT leaves or fruit. Our results suggest that the particle size does not affect iron chelating ability. Ganjewala et al. (2013) have determined the iron adsorption power of methanol extract leaf and fruit of Kadam. They found that leaf was higher than that of the fruit and the reason was that leaf has higher levels of phenolics in these tissues.

3.4. Reducing Power

Results of reducing power are presented in Figure 1C, similar to the results of iron chelating ability CTLP powder had higher reducing power than CTFP ($p<0.05$). The reducing power of CTLP with particle size less than 150 μm was higher than that of other treatments but slightly less than that of

ascorbic acid ($p<0.05$). However, at 1.0% (w/v), leaves powder had similar reducing power to fruit powder, regardless of particle size ($p>0.05$). In fruits, no difference in reducing power by particle size was observed, regardless of the concentration ($p>0.05$). The reducing power increased with increasing concentration for all particles ($p<0.05$) except for CTFP 150 μm ($p>0.05$). According to Natić et al. (2015) reported that reducing power has a positive correlation with total phenolic content. In this study, the values of the total phenolic content of CT leaves treatments showed higher than CT fruits treatments ($p<0.05$). Moreover, since CTLP contained more rutin (Table 3), the reducing ability of CTLP seems to higher than CTFP (Firuzi et al., 2005). Jeong et al. (2009) have reported that the act of reducing power of 100℃ water extract from different parts of CT tree showed high results in the order of leaves, fruit, root, and stem. Among various parts of CT tree extracted with hot water, leaves had an excellent ability to react with and stabilize active oxygen, similar to the results of the present study.

Experiment II: Product quality of pork patties with CT fruits powder

3.5. Individual antioxidant components form CTLF and CTFP

In order to establish CTLF and CLFP in terms of bioactive compounds and antioxidant activity in this study, they were analyzed by HPLC. The HPLC analysis of phenolic acids in CTLP and CTFP presented in Table 3. Comparison with individual antioxidant components including gallic aicd, ferulic acid, chlorogenic acid, rutin, isovanillic aicd allowed the extracted compounds to be detected and quantified by HPLC. Rutin and ferulic acid was detected in CTLP and CTFP. Out of the two phenolic compounds rutin was found in the highest concentration compared to ferulic acid amounting 191.56 mg / 100 g of dry sample in CTLP. In contrast, CTFP detected more ferulic acid than rutin. Rutin and ferulic acid both showed to contribute to the antioxidant activity of natural antioxidant resources and which have health benefits (Abarikwu et al., 2015). These results indicated that CTLP and CTFP could be a potential source of antioxidants.

3.6. pH and color of pork patties

Pork patties were prepared by adding 0.5 and 1.0 g/ 100 g of CTFP. Physicochemical properties and fatty acid compositions of pork patties were then measured during refrigerated storage. As shown in Table 4, the pH of pork patties with the addition of fruit powder did not interact between treatments and

storage periods ($p>0.05$). There were no significant ($p>0.05$) differences in the pH between CTFP-0 and treatments added with CTFP. As the storage period increased, the pH also increased for all treatments. Lee and Kim (2015) have shown that the pH and total acid of CT fruit are 6.05 and 0.31 mg/mL, respectively. Thus, the addition of CTFP did not affect the pH of pork patties in this study.

As shown in Table 4, there was no correlation between the amount of CTFP added and the storage period. Pork patties with CTFP had lower lightness (L *) values than the CTFP-0 and reference values. Redness (a *) and yellowness (b *) values were higher ($p<0.05$) as the amount of CTFP added was increased. On the other hand, there were no differences in lightness, redness, or yellowness during storage ($p>0.05$). According to Jo et al. (2017), when colors of ripe fruits of *Curdrania. Tricuspidata*, the values of L *, a *, and b * were 33.41, 26.80, and 16.57. Thus, the colors of pork patties were affected by the addition of CTFP. Yong (2015) has found that the red color of CT fruit is mainly induced by carotenoid pigment. The CT fruit was found to show the presence of four components. Among these components, antheraxanthin was identified. Sasaki et al. (2008) have investigated meat color changes after adding fucoxanthin, a significant carotenoid in algae, to patties of chicken breasts. They found that fucoxanthin decreased the lightness (L *), but increased redness (a *) and yellowness (b *). These results were similar to those of the present study.

3.7. Lipid oxidation inhibition of pork patties

As shown in Figure 2, interaction ($p<0.05$) between storage days and pork patty treatment was observed in the result of TBARS. From day 10, CTFP-0 had higher TBARS values than other treatments ($p<0.05$), the pork patties containing ascorbic acid, CTFP-0.5 and CTFP-1.0 treatments were not different ($p>0.05$). TBARS of CTFP-0 increased as the storage period increased. However, after adding CTFP and ascorbic acid, TBARS did not show any significant change for 14 days, indicated a delayed lipid oxidation activity ($p>0.05$). Results of TBARS of pork patties containing CTFP-0.5 and 1.0 for all storage periods showed no significant difference. The phenolic compound of ferulic acid and rutin in CTFP (table 3) have the ability to decrease the value of TBARS (Vaisali et al., 2016). Also, there are various Phenolic compounds in CTFP (Yong, 2015; Choi et al., 2009; Hong and Hong, 2015), that can lower lipid oxidation (Singleton et al., 1999). Tapp et al. (2012) have added noni (also called Indian mulberry) to beef patties at different concentrations and then measured TBARS during the storage period. As a result, it has been reported that the antioxidant capacity of noni can delay lipid oxidation,

thereby the expiration date could be prolonged.

Figure 3 shows the results of POV of pork patties added with 0.5 and 1.0 g/ 100 g of CTFP according to the storage period of pork patties. The POV showed an interaction between storage period and treatment ($p<0.05$). CTFP-0 showed no significant ($p>0.05$) difference in POV from the beginning of storage to 10 days after storage. However, it showed higher value at 14 days after storage. The group added with 1.0 g/ 100 g CTFP showed no significant ($p>0.05$) difference in POV from the 7th day to the 14th day, and the addition of CTFP-0.5 did not show any significant difference in POV from the beginning of storage to at 14 days after storage ($p>0.05$). CTFP-1.0 increased from the 7th day of storage to the 0th day of storage ($p<0.05$), but there was no change from 7th to 14th day of storage ($p>0.05$). CTFP-0 had significantly higher POV than other samples from the 3rd to the 10th day of storage ($p<0.05$). In contrast, no significant difference in POV was observed between pork patties added with the reference, CTFP-0.5 and CTFP-1.0 during the storage period ($p>0.05$). Thus, the ability of CTFP to inhibit peroxidation could be determined. Yong (2015) reported that much chlorogenic acid among the phenolic compounds in CT powder which was considered to have influenced the results of this study (Sasaki et al., 2010).

3.8. Microbiological quality of pork patties

Table 4 presents results of microbial counts of pork patties added with CTFP during 14 days of storage under 4°C in a refrigerator. There was no interaction between the two factors (treatment and storage day) ($p>0.05$). During storage, TPC and VRB were increased ($p<0.05$). However, the addition of CTFP did not show any antimicrobial effect among all treatments ($p>0.05$). There was no significant change in TPC or VRB until seven days after storage ($p>0.05$). However, counts were increased significantly at 10 and 14 days after storage ($p<0.05$). There was no significant difference in TPC and *Enterobacteriaceae* between CTFP-0 and patties added with CTFP. Thus, CTFP had no antimicrobial effect. Choi et al. (2009) have reported that methanol extracts of CT fruit have antimicrobial activities against *Staphylococcus aureus*, *Bacillus cereus*, and *Enterobacter aerogenes*. Notably, the extract from fruit had higher antimicrobial activity against *Staphylococcus aureus* than extracts from other parts. The reason for no antimicrobial effect of CTFP added to pork patties could be related to the use of dry powder instead of an extract with an organic solvent such as methanol and ethanol.

Conclusion

This study was carried out to evaluate the antioxidant activity of freeze-dried CT fruit, and oven-dried CT leaves according to particle size (500 μm, 150 μm). Physicochemical properties and antioxidant activities of pork patties added with CT fruit were also determined. Total phenolic content was higher in leaves than in fruits ($p<0.05$). There was no significant difference in the content of total phenolic according to particle size ($p>0.05$). In the antioxidant activity, DPPH radical scavenging activity of CT fruit was higher than that of CT leaves ($p<0.05$). Iron chelating ability and reducing power of CT leaves were also higher than those of CT fruit ($p<0.05$). Based on pH and color values measured after applying CT fruit particles of 500 μm or less at a concentration of 0.5 or 1.0 g/ 100 g to pork patties, there was no interaction between treatment and storage period. Results of lightness (L *) showed that samples without adding CT fruit had lower L * values than those added with CT fruit, while redness (a *) and yellowness (b *) values were increased for pork patties added with CT fruit ($p<0.05$). Therefore, since the redness increased as CT fruit was added to meat products, it was confirmed that it could be used as a natural colorant that can be applied to other meat products. Lipid oxidation was lower on treatments using CT fruit during storage, and this result was confirmed by a low value of TBARS and POV ($p<0.05$). Consequently, the antioxidative activity of freeze-dried CT fruit was lower than that of oven-dried CT leaves. Although CT fruit was added to pork patties at a low concentration of only 0.5 g/ 100 g, it showed a positive effect in retarding lipid oxidation and color. Thus, CT fruit might be useful as a natural antioxidant and colorant, so it is considered that further research on the development of meat products using CT fruit should be conducted.

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Table 1. The formulation of pork patties with sifted (≤ 500 μm) CTFP as affected by different concentrations

Ingredient	Treatments†			
	CTFP-0	REF	CTFP-0.5	CTFP-1.0
Raw meat (%)	78.5	78.5	78.5	78.5
Fat (%)	20.0	20.0	20.0	20.0
Salt (%)	1.50	1.50	1.50	1.50
AA (%)	-	0.10	-	-
CTFP (%)	-	-	0.5	-
CTFP (%)	-	-	-	1.0
Total	100.00	100.10	100.50	101.00

AA= ascorbic acid; CTFP= freeze-dried *Cudrania tricuspidata* fruit powders

†Treatments : CTFP-0 = control patties without CTFP; REF = reference patty added with AA at 0.1 g/ 100 g; CTFP-0.5 = treatment pork patty added with CTFP at 0.5 g/ 100 g; CTFP-1.0 = treatment pork patty added with CTFP at 1.0 g/ 100 g.

524 Table 2. Total phenolic content (g/100 g) of CTLP and CTFP

	Treatments *					
	CTLP	CTFP	CTLP 150 μm	CTFP 150 μm	CTLP 500 μm	CTFP 500 μm
Total phenolic content (g/ 100 g dry matter)	3.54±0.40[a]	1.85±0.15[c]	2.80±0.08[b]	1.49±0.08[c]	2.80 ±0.21[b]	1.91±0.29[c]

525 [a-c]Means with different superscripts in the same row are different ($p < 0.05$).

526 CTLP= oven-dried *cudrania tricuspidata* leaf powders; CTFP= freeze-dried *Cudrania tricuspidata* fruit
527 powders
528 *Treatments: CTLP = crude particle size of CTLP; CTFP = crude particle size of CTFP; CTLP 150 μm =
529 150 μm or less particle size of CTLP; CTFP 150 μm =150 μm or less particle size of CTFP; CTLP 500 μm
530 = 500 μm or less particle size of CTLP; CTFP 500 μm = 500 μm or less particle size of CTFP.
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533 Table 3. Individual antioxidant components of CTLP and CTFP

Treatment	Individual antioxidant components [1)]				
	Gallic aicd	Ferulic acid	Chlorogenic acid	Rutin	Isovanillic acid
CTLP	N.D *	91.81[b]	N.D *	191.56[b]	N.D *
CTFP	N.D *	185.45[a]	N.D *	21.24[a]	N.D *

534 [a-b] Mean with differnet susperscript letters in the same column indicate significant differences at $p<0.05$.

535 [*] N.D. : Not Detected

536 CTLP= oven-dried *cudrania tricuspidata* leaf powders; CTFP= freeze-dried *Cudrania tricuspidata* fruit
537 powders

538 [1)] mg/ 100 g extracted D.M. CTLP and CTFP.

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543 Table 4. pH, Hunter color, TPC and VRB of pork patties with CTFP during refrigerated storage

	Parameters					
	pH	L *	a *	b *	TPC	VRB
Treatment	**	**	**	**	NS	NS
Storage	**	NS	**	NS	**	**
Treatment ×Storage	NS	NS	NS	NS	NS	NS
Treatment[1)]						
CTFP-0	5.72±0.08[a]	59.4±2.07[a]	8.37±1.82[c]	8.45±0.92[c]	4.40±0.95[a]	3.74±1.13[a]
REF	5.65±0.09[b]	60.3±1.43[a]	8.74±1.02[c]	8.90±0.66[c]	4.26±1.06[a]	3.65±1.21[a]
CTFP-0.5	5.73±0.08[a]	55.5±2.55[b]	15.2±1.82[b]	13.2±1.34[b]	4.50±0.76[a]	3.90±0.97[a]
CTFP-1.0	5.72±0.08[a]	55.5±1.49[b]	18.1±2.81[a]	15.4±1.52[a]	4.78±0.83[a]	4.10±1.05[a]
Day						
0	5.60±0.07[d]	56.8±2.70[a]	13.8±5.28[a]	11.4±2.47[a]	3.91±0.38[c]	3.21±0.42[c]
3	5.67±0.05[c]	57.4±2.60[a]	12.9±5.19[a]	11.0±3.26[a]	3.80±0.27[c]	3.04±0.41[c]
7	5.74±0.07[ab]	57.5±2.94[a]	13.3±4.70[a]	12.1±3.68[a]	4.16±0.47[c]	3.49±0.77[c]
10	5.72±0.02[bc]	57.8±3.71[a]	12.4±4.61[ab]	11.2±2.84[a]	4.78±0.74[b]	4.21±0.95[b]
14	5.79±0.05[a]	58.9±2.78[a]	10.6±3.78[b]	11.7±4.01[a]	5.76±0.74[a]	5.28±0.86[a]

544

545 [a-b] Means with different superscripts within same row are different ($p<0.05$). NS: not significant,
546 **indicate $p<0.05$.
547

548 AA= ascorbic acid; CTFP= freeze-dried *Cudrania tricuspidata* fruit powders

549 [1)]Treatment: CTFP-0 = control patty; REF = reference patty added with AA at 0.1 g/ 100 g; CTFP-0.5
550 = treatment pork patty added with CTFP at 0.5 g/100 g; CTFP-1.0 = treatment pork patty added with
551 CTFP at 1.0 g/100 g

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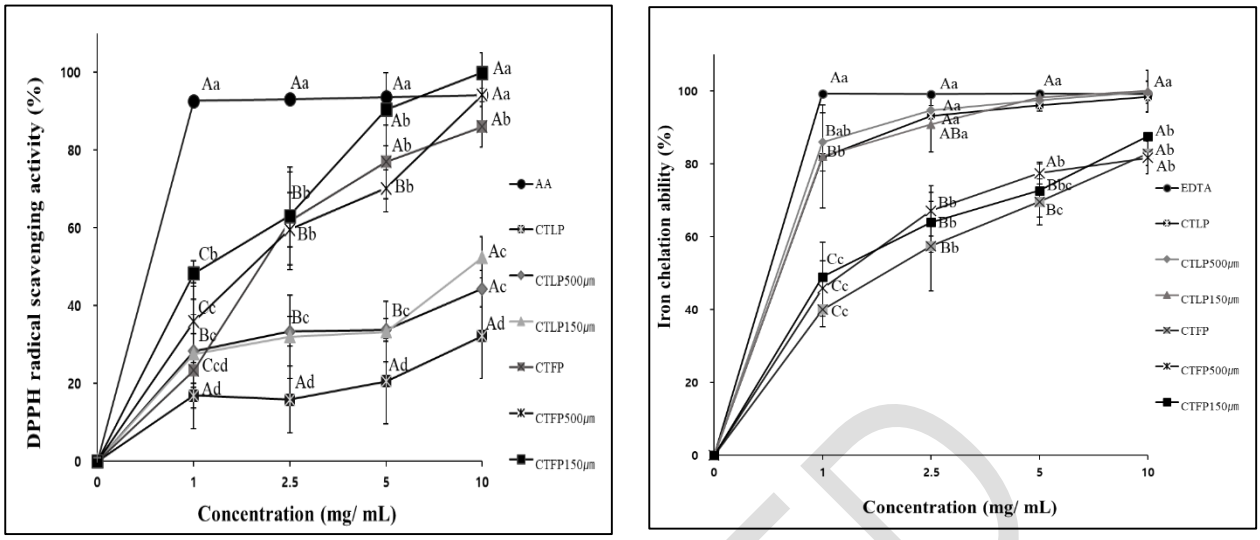
A **B**



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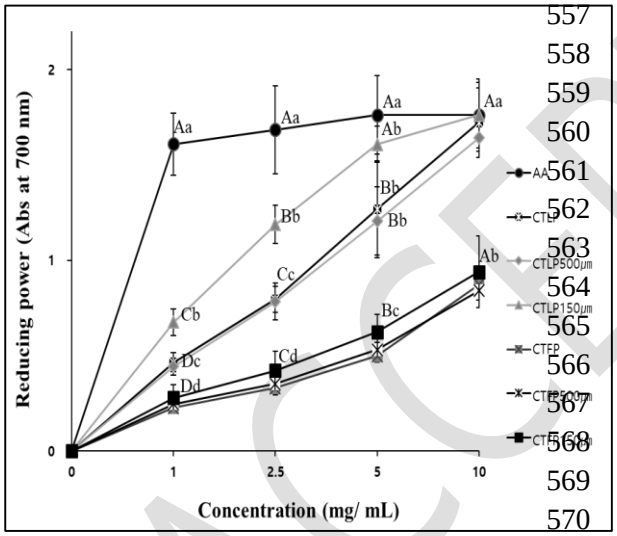


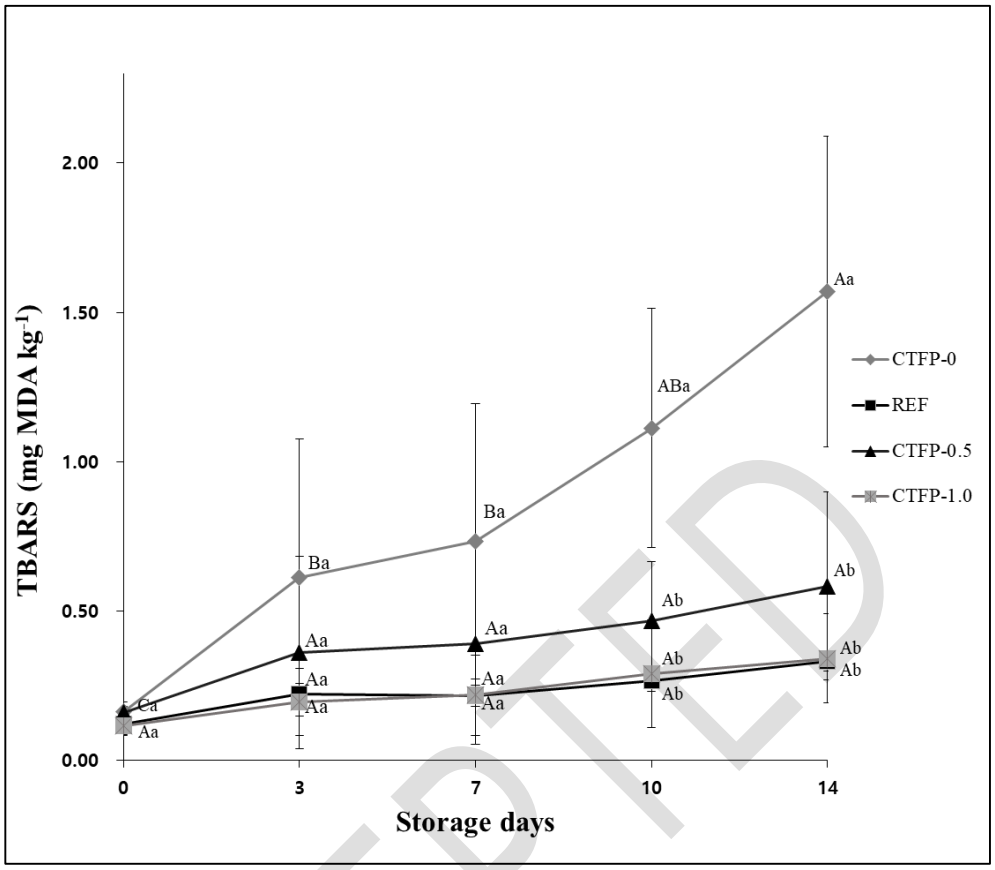
Figure 1. DPPH radical scavenging activity (%) of CTLP and CTFP (A). Iron chelating ability (%) of CTLP and CTFP (B). Reducing power (O.D) of CTLP and CTFP (C).

[A-D]Means with different superscripts in the same treatments are different ($p<0.05$).

[a-d]Means with different superscripts in the same concentration are different ($p<0.05$).

CTLP= oven dried *cudrania tricuspidata* leaf powders; CTFP= freeze dried *Cudrania tricuspidata* fruit powders

AA, ascorbic acid; CTLP, treatment crude particle size of CTLP; CTFP, treatment crude particle size of CTFP; CTLP 150 ㎛, treatment 150 ㎛ or less particle size of CTLP; CTFP 150㎛, treatment 150 ㎛ or less particle size of CTFP; CTLP 500 ㎛, treatment 500 ㎛ or more particle size of CTLP; CTFP 500 ㎛, treatment 500 ㎛ or more particle size of CTFP; EDTA, ethylenediaminetetraacetic acid.



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586 **Figure 2. TBARS of pork patties added with various level of CTFP during refrigerated storage.**
587 [A-B] Means with different letters within same treatments are different ($p<0.05$).
588 [a-c] Means with different letters within same storage days are different ($p<0.05$).
589 CTFP= freeze dried *Cudrania tricuspidata* fruit powders
590 Treatments are as shown in Table 1
591
592
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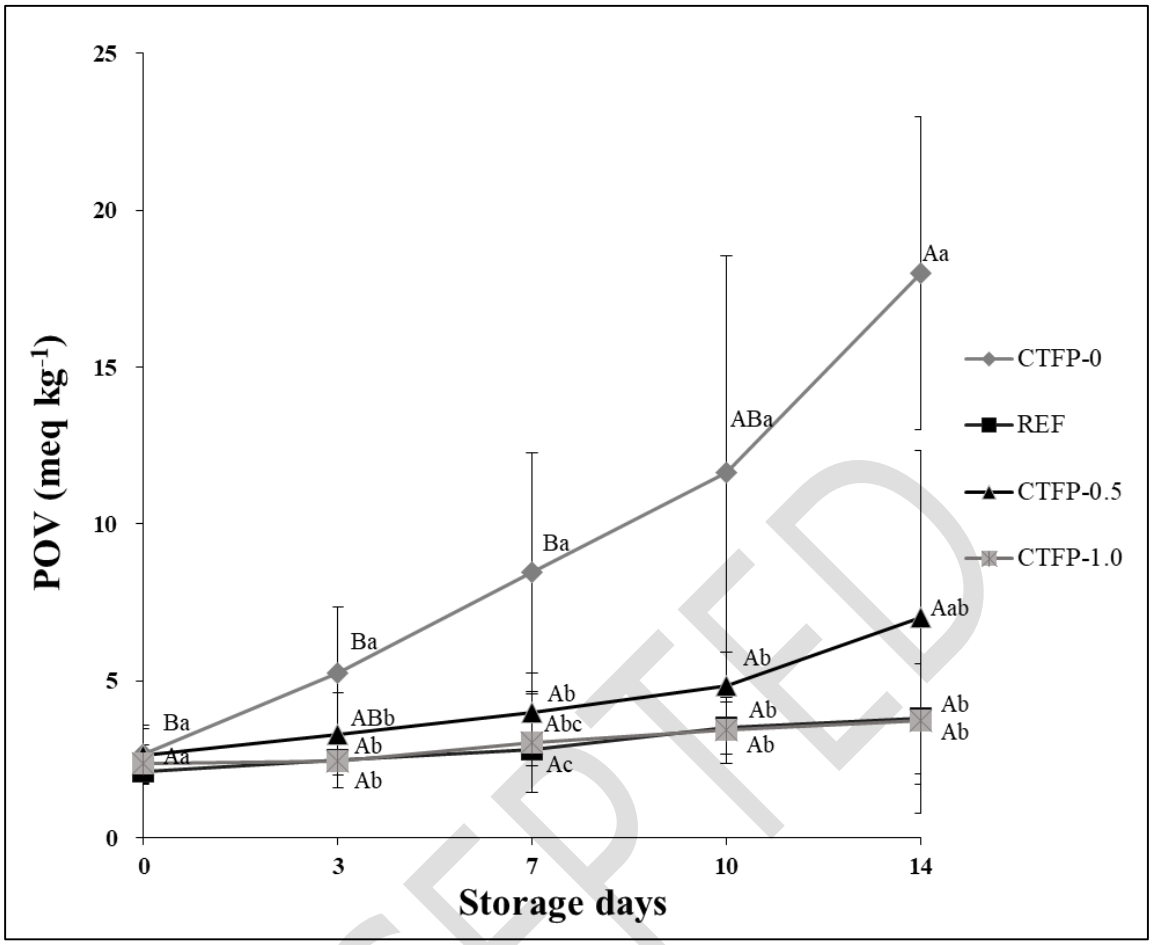

Figure 3. POV of pork patties added with various level of CTFP during refrigerated storage.

[A-B] Means with different letters within same treatments are different ($p<0.05$).

[a-c] Means with different letters within same storage days are different ($p<0.05$).

CTFP= freeze dried *Cudrania tricuspidata* fruit powders

Treatments are as shown in Table 1