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ARTICLE

Effect of Cacao Nip Extracts (CEs) on Quality Characteristics of Pork Patties during Cold Storage Period

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Abstract Cacao has been shown to have antioxidant effects and health benefits. However, the applicability of cacao as a meat preservative has not been thoroughly evaluated. Here, we examined the effects of cacao nib extracts (CEs) on suppression of fat oxidation and enhancement of quality characteristics of pork patties. Cacao nib powder was extracted in distilled water or 50%, 70%, or 99% ethanol. CEs prepared using 70% ethanol had the highest total phenolic and total flavonoid contents, and the highest 1,1-diphenyl-2-picrylhytdrazyl radical and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activities. Decompression-concentrated CEs prepared using 70% ethanol and 0.1% ascorbic acid were added to pork patties, and the physiochemical properties of the patties were measured. The pH of all pork patties increased during storage, but tended to decrease according to the CEs content. CEs enhanced the preservation of redness and texture of the pork patties during storage. Analysis of thiobarbituric acid reactive substances (TBARS) in patties revealed that fat oxidation was highly suppressed in all treatment groups containing CEs during storage, and TBARS values decreased according to CE content. Treatment with 0.1% CE reduced fat oxidation to a level similar to that of treatment with 0.1% ascorbic acid. Consumer flavor preference increased according to CE content, and overall preference was the highest for patties prepared with 0.05% and 0.075% CEs. Overall, 70% ethanol was found to be the optimal concentration for extraction of cacao nibs, and adding 0.05% or 0.075% CEs to pork patties yielded the highest quality.

Keywords cacao nib, antioxidant, pork patties, cacao extracts, refrigeration

Introduction

Meats are highly nutritious foods rich in proteins, lipids, vitamins, and minerals; however, the quality of nutritional components of meats and meat products may be reduced by chemical substances and microorganisms (Devatkal et al., 2014). Lipid

oxidation in meats can result in deterioration of meat quality, and the extent of deterioration is determined by chemical factors, such as the type of meat and oxygen content, and storage temperature (Kanner, 1994). Furthermore, because lipid oxidation affects the sensory quality of meat, including color, texture, and flavor, it also undermines the quality and consumer acceptability of meat products (Johnson and Decker, 2015). To prevent lipid oxidation, synthetic antioxidants, such as ascorbic acid, butylated hydroxyanisol, butylated hydroxytoluene, tert-butylhydroquinone, prophylgallate, nitrates, and nitrites, are added during meat production (Ribeiro et al., 2019; Shah et al., 2014). However, consumers have become increasingly concerned about the addition of synthetic antioxidants in processed meat products (Han and Ahn, 1998), which has instigated the use of natural antioxidants extracted from plants (Gil et al., 2001; Shah et al., 2014).

Natural antioxidants extracted from plants rich in phytochemicals (e.g., polyphenols, flavonoids) have been used to suppress lipid oxidation and improve quality characteristics of various meat products; indeed, the use of phytochemical-rich plant extracts has prolonged the shelf life of meat products and enhanced their quality (Mancini et al., 2015; Misumoto et al., 2005; Zhang et al., 2017). Experiments have been conducted using sausages containing curcumin extracts (Kim et al., 2007), pork patties containing brown soybean ethanolic extracts (Lee et al., 2016) and coffee extracts (Lise et al., 2004), Chinese-style sausages containing clove extracts (Zhang et al., 2017), and meat products containing rosemary extracts (Jin et al., 2016; Martinez et al., 2016) and green tea extracts (Mitsumoto et al., 2005; Ozvural et al., 2016).

Cacao (*Theobroma cacao* L.), the raw material used for making chocolate, is rich in phenolic compounds that can suppress lipid oxidation in meats. Cacao has been shown to reduce the risk of cardiovascular disease-related mortality and has antioxidant and antibacterial effects (Djouss et al., 2011; Ferrazzano et al., 2009; Lotito and Frei, 2006). Cacao nibs are peeled cacao beans that are used for preparing cocoa powder, cocoa butter, and chocolate products (Campos-Vega et al., 2018). Cacao extracts are a rich source of falvan-3-ols, such as catechins and epicatechins, and contain procyanidine and quercetin glycosides (Lamuela-Raventos et al., 2013; Sanchez-Rabaneda et al., 2003). Furthermore, cacao contains methylxanthine alkaloid, theobromine, and small amounts of quercetin derivatives (Cadiz-Gurrea et al., 2014). *In-vivo* and *in-vitro* studies have confirmed the bioactivities of cacao phenolic compounds (Djouss et al., 2011; Ferrazzano et al., 2009; Lotito and Frei, 2006). Ribas-Agusti et al. (2014) evaluated the effects of adding cacao extracts to sausages on meat quality. However, additional studies are still needed.

In this study, we examined the optimal ethanol concentration for extracting cacao nibs and assessed the effects of addition of cacao nib extracts (CEs) to pork patties on suppression of lipid oxidation and enhancement of meat quality during refrigerated storage for 15 days.

Materials and Methods

Analysis of antioxidant activity of cacao nibs

Preparation of CEs and evaluation of extraction yields

The cacao (*Theobroma cacao* L.) beans used in this study were produced in Ecuador and were purchased from TreeToBar (Namyangju-si, Korea). After roasting raw cacao beans at 180℃ for 15 min in a roaster (CNR-101A, Genesis, Ansan, Korea), cacao nibs, i.e., the bits of cacao beans obtained after the husk is peeled, were ground in an ultrafine grinder (JP5063-1, MHK, Osaka, Japan). The ground nibs were passed through a mesh (test sieve no. 35, Chunggye Co., Gyeonggi, Korea) to prepare cacao nib powder (500 μm). Cacao nib powder (20 g) was added to 200 mL of distilled water (DW) or 50%, 70%, or 99% ethanol and extracted in a shaker at 25℃ for 24 h. The CEs were filtered using filter papers (Whatman No. 2, GE

Healthcare, Little Chalfont, UK) and evaporated with a rotary evaporator (N-1200A, EYELA, Shanghai, China) at a temperature of less than 50°C. After evaporation, the weight of the CE was divided by the weight of the original cacao nib powder to calculate the extraction yield (%). To measure phenolic compounds and antioxidant activity, CE was dissolved in DW or 50%, 70%, or 99% ethanol (cat no. 64-17-5, ethyl alcohol anhydrous, DaeJung, Namyangju, Korea).

Analysis of total phenolic compounds (TPC)

TPC were measured using the Folin–Ciocalteu method with modifications (Slinkard and Singleton, 1977). Briefly, 2.4 mL of DW and 0.15 mL of 0.25 N Folin–Ciocalteu reagent were added to 0.15 mL of sample diluent (1 g CE/100 mL DW), vortexed, and reacted for 3 min. Next, 0.3 mL of 1 N Na_2CO_3 was added, samples were left to stand in the dark for 2 h, and the absorbance at 725 nm was measured. Tannic acid was used as a standard substance and expressed as mg tannic acid equivalent (TAE) per g cacao nib powder.

Analysis of total flavonoid compounds (TFC)

Total flavonoid compounds (TFC) were measured using the Davis method (Um and Kim, 2007). Briefly, 10 mL of 90% diethylene glycol and 1 mL of 1 N NaOH were added to 1 mL of sample diluent, vortexed, and reacted in a water bath (10.10 ESI/SK, Alto Shaam, Menomonee Falls, WI, USA) at 37°C for 1 h. Then, the absorbance at 420 nm was read. Quercetin acids was used as a standard substance, and TFCs were expressed as mg quercetin acids per g cacao nib powder.

1,1-Diphenyl-2-picrylhytdrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was evaluated using the method of Brand–Williams et al. (1995), with modifications. Briefly, 1 mL of sample diluent was added to 4 mL of 4×10^{-4} M DPPH solution. The reaction mixture was left to stand in the dark for 30 min. Then, the absorbance at 517 nm was read, and the DPPH scavenging activity of the CEs was calculated according to the following equation:

$$\text{Scavenging activity (\%)} = [1 - (\text{Sample absorbance} / \text{Control absorbance})] \times 100$$

2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radical scavenging activities

$ABTS^+$ radical scavenging activity was measured using the method described by Cuong et al. (2016), with modifications. Briefly, 7.0 mM $ABTS^+$ solution in DW and 2.45 mM $K_2O_8S_2$ solution in DW were mixed and left to stand in the dark for 16 h. This solution was then diluted with ethanol. One milliliter of sample diluent was added to 9 mL of $ABTS^+$ solution. After mixing, the absorbance at 734 nm was read six times at 1-min intervals, and the average value was used. The $ABTS^+$ scavenging activity of the CEs was calculated according to the following equation:

$$\text{Scavenging activity (\%)} = [1 - (\text{Sample absorbance} / \text{Control absorbance})] \times 100$$

Processing of pork patties with different amounts of CEs (dried 70% ethanol extracts)

The mixing ratios of the ingredients are shown in Table 1. The chilled pork loin obtained within 24 h after slaughter (Hongju Meat Co., Hongseong, Korea). Raw meat and back fat were crushed using a grinding machine (PA-82, Mainca,

Table 1. Formulation of pork patties with different amounts of cacao nips extracts

Ingredients (%)		CON	CE-0.025	CE-0.05	CE-0.075	CE-0.1	ASC-0.1
Main	Meat	65	65	65	65	65	65
	Back fat	25	25	25	25	25	25
	Water	10	10	10	10	10	10
Additive	Salt	1.5	1.5	1.5	1.5	1.5	1.5
	CE	-	0.025	0.05	0.075	0.1	-
	Ascorbic acids	-	-	-	-	-	0.1

CE, cacao nip dry extracts with 70% ethanol and concentrated under reduced pressure.

Barcelona, Spain) equipped with a 3-mm plate. All ingredients were mixed using kneader (K5SS, KichenAid Co., Benton Harbor, MI, USA). The pork patty was manufactured using a conventional patty maker (1 cm in thickness and 8.5 cm in diameter). The pork patties were baked in a convection oven at 80°C for 30 min. After baking, the pork patties were stored in a refrigerator at 4°C for 15 days.

pH and color measurements

The pH of the pork patties was measured using a pH meter (Coring 340, Mettler Toledo, Schwerzenbach, Switizerland) in a homogenate of 5 g sample and 20 mL of DW (PT 2500 E; Kinematica AG, Kusnacht, Switzerland).

The color values were measured directly on the patty surface using a Hunter color reader (CR-400, Minolta, Osaka, Japan). The results are expressed as CIE L*, CIE a*, and CIE b*. A white standard plate with a lightness of +94.65, redness of –0.43, and yellowness of +4.12 was used as the reference.

Texture profile analysis

The texture of the patties was analyzed using a texture analyzer (TA-XT2, Stable Micro System, Haslemere, UK) equipped with a round probe (75 mm diameter). Samples (2.5×2.5×1 cm) were cut from the central portion of each pork patty. The texture features analyzed included hardness, springiness, cohesiveness, chewiness, and gumminess. The conditions of texture analysis were as follows: test speed, 3.0 mm/s; post-test speed, 5.0 mm/s; pre-test speed, 5.0 mm/s; trigger force, 5 g; test distance, 7.0 mm. The texture of pork patties was analyzed 10 times per replication.

Analysis of thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances were determined by distillation. Briefly, 10 g of the heated sample was homogenized for 3 min at 1,000×g using a homogenizer (AM-5, Nihonseiki Kasha, Osaka, Japan). The sample was then mixed with 2.5 mL of HCl, 3–4 boiling stones, 1 mL of defoamer, and 47.5 mL of DW. The samples were purified in a distiller (MS-E102, MTOPS, Seoul, Korea) for 10 min. Next, 5 mL of the collected distillate and 5 mL of TBA reagent were mixed and heated in a constant-temperature water bath (JSWB-30T, JSR, Seoul, Korea) at 100°C for 35 min. After cooling for 10 min, the absorbance at 538 nm was read using a spectrophotometer (Libra S22, Biochrom, Nottingham, UK). The measured value was multiplied by a factor value of 7.8 to represent the TBARS content.

Total microbial plate counts

All instruments and solutions used in microbiological experiments were sterilized before use. To determine total aerobic bacterial count for patties, 10 g sample and 90 mL of 0.1% peptone water (Difco, Becton, NJ, USA) were transferred into a sterile stomacher bag and homogenized (HG300V, MAYO, Milano, Italy) for 3 min. Serial dilutions of the homogenate were prepared with 0.1% peptone water. Next, 0.1 mL of liquid was spread on plate count agar. The total plate count was determined after incubation at 37°C for 24–48 h. Microbial colonies were counted using a digital colony counter (KT0074A, S&N, Seoul, Korea) and were expressed as Log CFU/g patty sample.

Sensory evaluation

Each sample was evaluated by sensory panelists twice. Twenty panelists were screened from 25 potential panelists based on a basic taste identification test. Each sample was scored on a single sheet, using a 9-point descriptive scale (with 1 being extremely undesirable and 9 being extremely desirable). The pork patties were evaluated for color, flavor, tenderness, juiciness, and overall acceptability.

Statistical analysis

All experiments were repeated at least three times. The data are expressed as means±SDs. The data were analyzed by one-way analysis of variance and Duncan's multiple range post-hoc tests using SPSS v. 25.0 (IBM, Armonk, NY, USA). Differences with p values of less than 0.05 were considered significant.

Results and Discussion

Antioxidant activities of CEs prepared using different ethanol concentrations

CE yields

The extraction yields of CEs prepared using different ethanol concentrations are shown in Fig. 1. The extraction yield tended to decrease as the ethanol concentration increased; the lowest yield was observed for 99% ethanol, and the highest

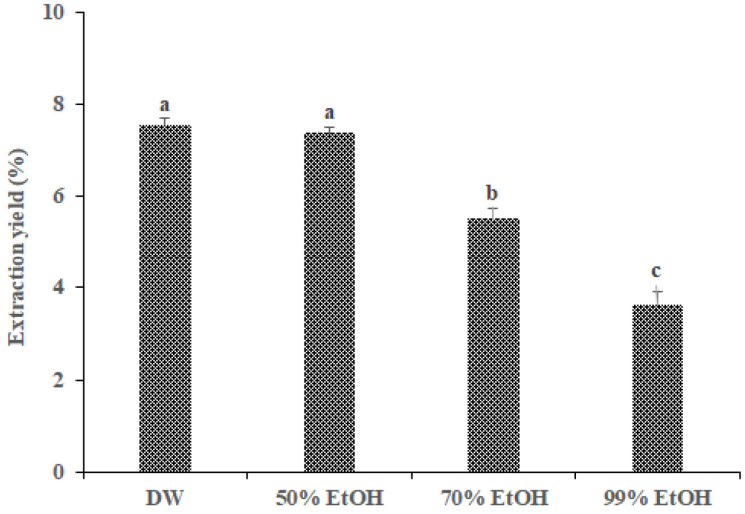

Fig. 1. Extraction yield of cacao nips extracts by different levels of ethanol. [a–c] Mean sharing different letters are significantly different ($p<0.05$).

yields were observed for DW and 50% ethanol ($p<0.05$). In general, water-soluble polymeric materials such as starch, fiber, pectin, and proteins have higher extraction yields when extracted with watery solutions than when extracted with ethanol. When using a high concentration of ethanol, glycosides, organic acids, and essential oils are extracted. Thus, the choice of extraction solvent is determined by the material to be extracted (Lee et al., 2009). Therefore, our findings can be explained by the fact that more water-soluble components, such as sugars, proteins, and alkaloids were extracted from the cacao nibs when using DW and 50% EtOH. Similarly, Lee et al. (2016) reported that the extraction yield was higher with 75% and 50% ethanol then when 95% ethanol was used. Furthermore, Kim et al. (2004) reported that extraction yields for cacao bean husk were higher when a diluted ethanol solvent was used than when a pure ethanol solvent was used, similar to our findings.

TPC and TFC

As shown in Fig. 2, TPC and TFC were significantly different ($p<0.05$) in the CEs prepared with different ethanol concentrations. TPC and TFC were the highest in CE prepared with 70% ethanol and the lowest in CE prepared with 99% ethanol ($p<0.05$). The CE extraction yield increased with decreasing ethanol concentration of the solvent (Fig. 1); however, TPC and TFC yields decreased with decreasing ethanol concentration of the solvent (except 99% EtOH). Antioxidants present in plants are mostly polar, and the higher the ethanol concentration, the better the antioxidant extraction (Lee et al., 2016). However, the use of pure ethanol did not result in the highest antioxidant extraction yield because the soluble properties of phenolic compounds and flavonoid glycosides differ in polar and water-soluble solvents. Phenolic compounds, such as lignins, are ester-bound to cell wall components in the plant; thus, there is an insoluble fraction in the alcohol, whereas glycosides are soluble in alcohol. Flavonoids, on the other hand, are polar and soluble in alcohols, whereas glycosides are relatively soluble in water (Woo et al., 1977). Therefore, to extract antioxidant compounds from plants, aqueous ethanol mixtures are used rather than pure ethanol (Dai and Mumper, 2010). For efficient antioxidant extraction, it is important to optimize the extraction conditions in view of homeostasis of the target compound and the concentration of the antioxidant (Shah et al., 2014). In most studies on cacao extracts, aqueous 50%–80% polar solvents resulted in the best antioxidant extraction yields (Kim et al., 1993; Kim et al., 2004; Wang and Helliwell, 2001). In our study, TPC and TFC yields were the highest when extracted with 70% ethanol.

On the other hand, Hu et al. (2016) reported that the TPC contents of extracts from fat-removed cacao beans using 70% aqueous methanol at 40°C for 2 h ranged from 36.21 to 55.58 mg GAE/g sample depending on the roasting conditions, and

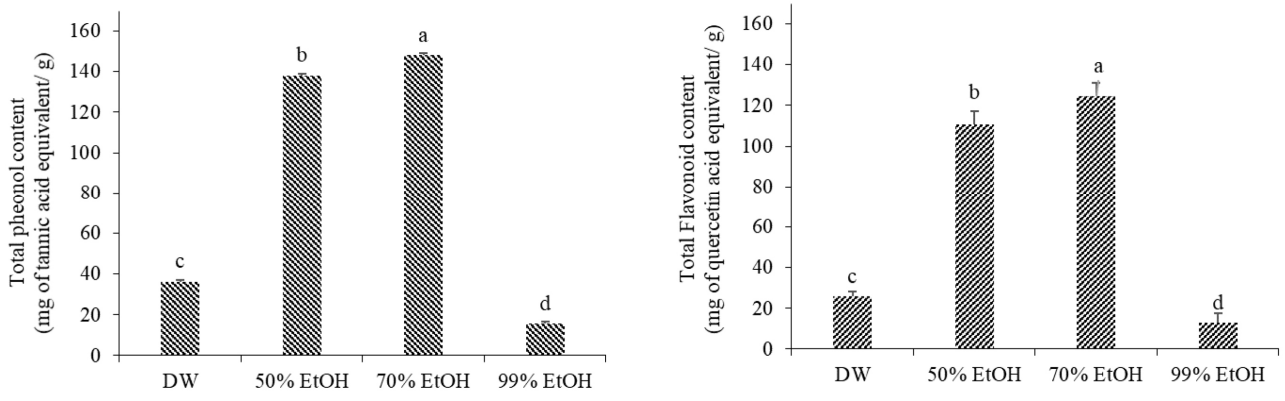

Fig. 2. Total phenol and flavonoid contents of cacao nip extracts (CEs) by different levels of ethanol. [a–d] Mean sharing different letters are significantly different ($p<0.05$).

the TFC contents ranged from 15.55 to 38.07 mg catechin/g sample. However, in this study, 70% ethanol yielded the highest TPC (148.09 mg TAE/g) and TFC (124.59 mg QEA/g) contents ($p<0.05$). These results may be related to the presence of phytochemicals, such as methylxanthine, alkaloid, and theobromine, in addition to catechins in cacao nibs (Cadiz-Gurrea et al., 2014). Additionally, the efficiency of extracting phenolic compounds from plants varies depending on sample preprocessing, sample ratio, solvent type, extraction temperature, and extraction time (Casazza et al., 2011).

DPPH and ABTS$^+$ radical scavenging activities

The DPPH and ABTS$^+$ radical scavenging activities of the CEs are shown in Fig. 3. In both DPPH and ABTS$^+$ radical scavenging assays, ascorbic acid had the highest antioxidant activity, followed by CEs prepared using 70% ethanol, 50% ethanol, DW, and 99% ethanol ($p<0.05$). This was generally consistent with the TPC and TFC results in our study. Dudonne et al. (2009) reported that TPC contents were highly correlated with DPPH and ABTS$^+$ radical scavenging. This is because phenolic compounds have aromatic rings with hydroxyl groups and can therefore remove free radicals by forming phenoxyl radicals (Bors and Michel, 2002). Accordingly, we concluded that 70% ethanol was the most effective for extracting antioxidants from cacao nibs; thus, we used 70% ethanol as the extraction solvent in subsequent experiments.

Physicochemical properties of pork patties containing CEs during refrigerated storage

pH and color measurements

Table 2 shows changes in the pH of pork patties containing CEs during storage. The pH tended to increase during storage in all treatment groups ($p<0.05$), suggesting that the production of ammonia, amine, and other alkaline substances caused by bacterial activities and protein deamination during storage alkalized the pork patties (Nychas et al., 1998). In line with our findings, multiple studies have demonstrated that the pH of meat products tends to increase during storage (Emin et al., 2016; Mancini et al., 2015; Rodriguez-Calleja et al., 2005). In contrast, the pH tended to decrease as the CE content increased during storage, and treatment with 0.1% ascorbic acid resulted in the lowest pH in all samples ($p<0.05$). According to Miller et al. (1980), the pH of meat products is determined by the ratio of base meat and additives and affects freshness, moisture

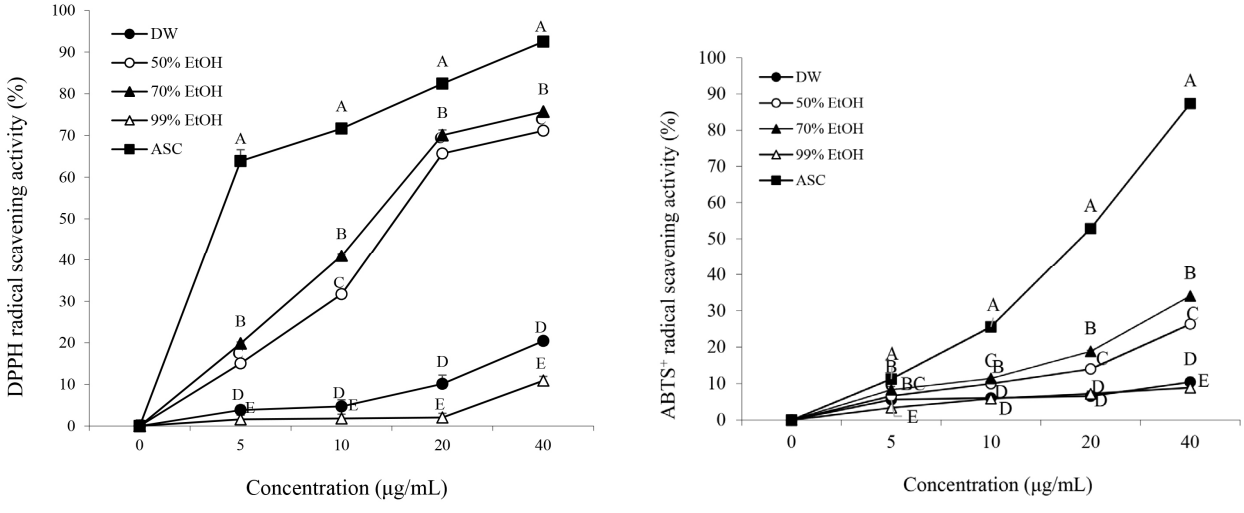

Fig. 3. DPPH and ABTS$^+$ radical scavenging activity of cacao nip extracts by different levels of ethanol. $^{A–E}$ Mean within the same concentration with different letters are significantly different ($p<0.05$).

Table 2. Changes in pH values and color values of cooked patties formulated with different amounts of cacao nip extracts (CEs) during cold storage (4°C)

Traits	Storage periods (d)	CON	Treatment[1)]				
			CE-0.025	CE-0.05	CE-0.075	CE-0.1	ASC-0.1
pH	0	5.79±0.01^{Da}	5.78±0.01^{Bab}	5.76±0.02^{Cb}	5.74±0.01^{Dc}	5.73±0.02^{Cc}	5.63±0.03^{Dd}
	3	5.84±0.01^{Ca}	5.81±0.01^{Bb}	5.80±0.01^{Bb}	5.77±0.01^{Cc}	5.77±0.02^{Bc}	5.72±0.02^{Cd}
	7	5.84±0.01^{BCa}	5.82±0.01^{Bab}	5.81±0.01^{Bbc}	5.80±0.03^{Bcd}	5.78±0.03^{Bd}	5.74±0.01^{BCe}
	10	5.86±0.03^{ABa}	5.85±0.01^{Aa}	5.83±0.01^{Ab}	5.81±0.02^{ABb}	5.78±0.01^{Bc}	5.75±0.01^{Bd}
	15	5.87±0.01^{Aa}	5.85±0.02^{Aab}	5.84±0.01^{Abc}	5.83±0.02^{Ac}	5.81±0.02^{Ad}	5.78±0.02^{Ae}
CIE L*	0	66.45±0.75^{Ca}	65.24±0.52^{Bb}	64.67±1.15^{bc}	62.97±1.29^{Bc}	58.84±1.06^{Db}	67.78±0.86^{a}
	3	66.82±0.44^{Cab}	66.34±1.57^{ABab}	65.33±1.19^{bc}	63.19±0.62^{ABc}	60.20±2.36^{ABd}	68.49±1.34^{a}
	7	67.35±0.86^{BCa}	66.23±1.43^{ABa}	65.51±0.62^{ab}	62.76±2.38^{Bbc}	61.70±1.86^{Ac}	67.96±1.17^{a}
	10	68.74±1.07^{ABa}	66.68±0.64^{ABb}	64.55±0.60^{c}	63.92±1.11^{ABc}	61.73±0.67^{Ad}	69.48±0.28^{a}
	15	69.63±1.54^{Aa}	67.98±1.02^{Aa}	65.35±0.83^{b}	65.43±0.75^{Ab}	60.93±0.48^{ABd}	68.46±0.87^{a}
CIE a*	0	4.61±0.37^{Ac}	4.89±0.41^{Ac}	5.55±0.13^{Ab}	5.76±0.21^{ab}	6.03±0.19^{Aa}	5.45±0.19^{Ab}
	3	4.02±0.18^{Bb}	4.56±0.21^{Ac}	4.92±0.16^{Bb}	5.62±0.09^{b}	6.05±0.17^{Aa}	5.22±0.32^{ABbc}
	7	3.59±0.33^{Bc}	3.62±0.27^{BCc}	4.47±0.13^{BCb}	5.53±0.36^{ab}	5.81±0.26^{Aba}	5.19±0.22^{ABb}
	10	2.76±0.19^{Cd}	3.24±0.45^{BCc}	4.14±0.15^{Cb}	5.39±0.26^{a}	5.62±0.35^{Ba}	5.18±0.28^{ABa}
	15	2.26±0.45^{Cd}	2.98±0.53^{Cc}	3.23±0.66^{Dc}	5.19±0.43^{a}	5.61±0.30^{Ba}	4.85±0.22^{Ba}
CIE b*	0	14.42±0.54^{BCa}	13.72±0.39^{a}	13.60±0.27^{ABb}	13.99±0.57^{Ba}	13.83±0.45^{ABa}	11.19±0.70^{Cb}
	3	14.04±0.86^{Ca}	13.64±0.21^{a}	14.00±0.25^{Aab}	12.44±0.90^{Bbc}	12.71±1.12^{Babc}	11.97±0.58^{Cc}
	7	15.07±0.27^{BCa}	14.35±0.41^{ab}	14.73±0.63^{Aa}	12.21±0.51^{Bc}	12.74±0.43^{Bc}	13.55±0.33^{Bb}
	10	15.23±0.23^{Aba}	14.11±0.28^{b}	14.06±0.43^{Ab}	13.79±0.76^{Ab}	12.90±0.30^{ABc}	13.86±0.77^{ABb}
	15	15.74±0.79^{Aa}	14.23±0.86^{bc}	14.74±0.92^{Abc}	13.01±0.64^{ABc}	13.92±0.36^{Abc}	14.75±0.49^{Aab}

[1)] The mixing ratios of the ingredients are shown in Table 1.
$^{A–D}$ Means within a column with different letters are significantly different ($p<0.05$).
$^{a–d}$ Means within a row with different letters are significantly different ($p<0.05$).

retention, meat color, and texture. The pH (5.68) of the CE solution used in the current study and the acidity of ascorbic acid were thought to have affected the pork patties.

As shown in Table 2, lightness and yellowness of the patties significantly decreased, whereas red tended to increase, with increasing CE content ($p<0.05$, 0 d). During the roasting of cacao nibs, melanoidin is formed in amino-carbonyl reactions (Maillard reactions) (Oliviero et al., 2009). Melanoidin modifies the color, taste, and flavor of the cacao nibs, specifically, it increases darkness and red color (Choi et al., 2016). The color values of CEs are CIE L* 35.61, CIE a* 9.55, and CIE b* 10.51, indicating that the cacao nib pigments affect patty color. During storage, lightness and yellowness of all patties increased, whereas redness decreased ($p<0.05$). However, the decrease in redness was suppressed with increasing CE content. In the control, redness decreased from CIE a* 4.61 (0 d) to CIE a* 2.26 (15 d), whereas for 0.1% CE treatment, redness decreased from CIE a* 6.03 (0 d) to CIE a* 5.61 (15 d). On day 15, the highest redness was observed for samples treated with 0.075% CE, 0.1% CE, or 0.1% ascorbic acid, whereas the control group had the lowest redness value. Choe et al. (2011) reported that reductions in redness in stored meats are related to the production of brown metmyoglobin as a result of

myoglobin oxidation, and numerous studies have reported that ascorbic acid effectively delays the oxidation-related discoloration in ground pork and beef through antioxidant activities (Ahn and Nam, 2004; Banon et al., 2007; Mitsumoto et al., 2005). In line with our findings, Schilling et al. (2018) reported that the antioxidant effects of rosemary and green tea extracts preserved the redness of sausages by inhibiting the formation of primary and secondary lipid oxidation products, which facilitate the oxidation of myoglobin. Thus, the abundance of phenolic compounds in cacao nibs seemed to suppress the discoloration of patties by blocking lipid oxidation during storage, similar to ascorbic acid.

Texture profile analysis

As shown in Table 3, hardness and chewiness decreased, whereas gumminess increased with increasing CE content ($p<0.05$, 0 d). Hardness tended to increase in all experimental groups during storage; however, compared with the control, the increase in hardness was not as dramatic in the CE- and 0.1% ascorbic acid-treated groups ($p<0.05$). In particular, the hardness score in the control group increased from 3.21 (0 d) to 4.09 (15 d), whereas that in the 0.1% CE group increased only slightly from 2.09 (0 d) to 2.17 (15 d). Adding antioxidants to meat products to protect the fascia from lipid oxidation preserves the membrane integrity of muscle fibers, thereby preventing loss of moisture in muscle fibers and textural changes of meat products (Estevez et al., 2006; Mitsumoto et al., 1995). Zhang et al. (2003) reported that sage, a natural antioxidant spice, delays changes in the hardness of sausages during refrigerated storage by inhibiting lipid oxidation and protein oxidation. The changes in hardness, chewiness, and gumminess during refrigerated storage in sausages containing clove extracts, as reported by Zhang et al. (2017), were similar to the changes observed in our study. Therefore, this study confirmed that CEs can delay texture degradation in pork patties.

TBARS

Table 4 shows the TBARS contents of pork patties during refrigerated (4°C) storage. TBARS significantly increased in all groups during storage ($p<0.05$), suggesting that fats in pork patties auto-oxidize, even during refrigerated storage (Wenjiao et al., 2014). In CE-containing patties, TBARS contents were 0.15–0.29 mg MDA/kg meat on day 0, which was substantially lower than that in the control group (0.54 mg MDA/kg; $p<0.05$). The TBARS contents of the control group markedly increased during storage, from 1.21 mg MDA/kg (3 d) to 1.98 mg MDA/kg (15 d; $p<0.05$). However, TBARS decreased with increasing CE contents ($p<0.05$). In particular, the ascorbic acid treatment group showed the lowest TBARS content during storage. Ascorbic acid suppresses lipid oxidation by H_2O_2 and hydroxyl radicals and therefore is used as an additive to prevent lipid oxidation in meat products (Ribeiro et al., 2019; Sarma et al., 1997). Moreover, 0.1% CE suppressed lipid oxidation during storage to a level similar to that observed for 0.1% ascorbic acid ($p<0.05$). These findings suggested that CEs effectively suppress lipid oxidation during refrigerated storage.

Lipids in meats and meat products are easily oxidized, and the resulting hydroperoxides produce secondary lipid oxidation products, i.e., aldehydes and ketones, which cause off-flavor, thereby diminishing quality (Cuong and Chin, 2016). Antioxidants can prevent lipid peroxidation by disrupting the chain reaction through the elimination of radical formation and breakdown of peroxides or by reducing the local oxygen concentration (Dorman et al., 2003). Cacao nibs contain adequate amounts of polyphenolic compounds, which are natural antioxidants, particularly the monomers catechin and epicatechin and the dimer procyanidin (Lamuela-Raventos et al., 2005; Martinex et al., 2012). The current study confirmed that CEs are abundant in phenolic compounds and have high radical-scavenging activities. Jayawadana et al. (2011) and Tackahata et al. (2001) reported that soybean extracts containing procyanidin had high DPPH radical scavenging activity and delayed MDA

Table 3. Texture profile analysis values of cooked patties formulated with different amounts of cacao nips extracts (CEs) during cold storage (4℃)

Traits	Storage periods (d)	CON	Treatment[1)]				
			CE-0.025	CE-0.05	CE-0.075	CE-0.1	ASC-0.1
Hardness (kg)	0	3.21±0.32^{Ca}	2.56±0.31^{bc}	2.51±0.18^{Bbcd}	2.26±0.17^{Bcd}	2.09±0.44^{Bd}	2.76±0.10^{Bb}
	3	3.54±0.34^{BCa}	2.89±0.26^{c}	2.36±1.69^{Bd}	2.61±0.25^{Ac}	2.05±0.25^{Bd}	3.09±0.22^{Ab}
	7	3.53±0.17^{BCa}	2.75±0.43^{b}	2.40±0.37^{Bbc}	2.15±0.15^{Bc}	2.74±0.31^{Ab}	2.44±0.15^{Bb}
	10	3.97±0.28^{ABa}	2.79±0.37^{bc}	2.66±0.42^{Abc}	2.58±0.54^{Ac}	2.74±0.26^{Ac}	3.16±0.26^{Ab}
	15	4.09±0.40^{Aa}	2.81±0.23^{b}	2.64±0.35^{ABbc}	2.52±0.29^{ABbc}	2.17±0.30^{ABc}	2.92±0.16^{ABb}
Chewiness (kg)	0	2.07±0.10^{a}	1.14±0.17^{c}	1.09±0.08^{c}	1.09±0.08^{c}	1.05±0.24^{ABc}	1.57±0.44^{Ab}
	3	2.04±0.34^{a}	1.05±0.19^{bc}	0.90±0.09^{c}	1.11±0.23^{bc}	1.53±0.65^{Ab}	1.32±0.13^{ABb}
	7	1.50±0.24^{a}	1.02±0.23^{b}	0.95±0.19^{b}	1.07±0.14^{b}	1.16±0.16^{ABb}	1.02±0.62^{Bb}
	10	2.07±0.29^{a}	0.97±0.17^{c}	1.01±0.25^{bc}	1.09±0.13^{bc}	1.14±0.11^{ABbc}	1.33±0.16^{ABb}
	15	1.87±0.36^{a}	0.99±0.14^{bc}	0.89±0.19^{bc}	0.86±0.06^{bc}	0.76±0.12^{Bc}	1.27±0.49^{ABb}
Cohesiveness (ratio)	0	0.64±0.02^{Aa}	0.57±0.02^{Ac}	0.60±0.02^{Abc}	0.60±0.02^{Abc}	0.60±0.03^{Ab}	0.59±0.01^{Abc}
	3	0.62±0.05^{Aba}	0.52±0.03^{Bb}	0.52±0.03^{Bb}	0.54±0.03^{Bb}	0.56±0.04^{Bab}	0.51±0.02^{Bb}
	7	0.51±0.05^{Ba}	0.49±0.05^{Cbc}	0.51±0.03^{Bc}	0.53±0.03^{Babc}	0.54±0.03^{Bab}	0.49±0.02^{Bbc}
	10	0.58±0.04^{ABa}	0.44±0.04^{Bc}	0.46±0.04^{Bb}	0.49±0.02^{BCb}	0.52±0.03^{BCb}	0.49±0.04^{Bb}
	15	0.54±0.08^{Ba}	0.46±0.04^{BCab}	0.46±0.05^{Bb}	0.47±0.03^{Cab}	0.48±0.02^{Cab}	0.50±0.01^{Bab}
Springiness (cm)	0	0.79±0.02^{ABab}	0.78±0.05^{Abc}	0.73±0.03^{c}	0.83±0.02^{Aa}	0.84±0.03^{Aa}	0.85±0.01^{a}
	3	0.77±0.03^{Bb}	0.69±0.02^{Bc}	0.74±0.03^{c}	0.81±0.02^{ABab}	0.83±0.05^{ABab}	0.84±0.03^{a}
	7	0.79±0.03^{ABab}	0.76±0.05^{ABb}	0.77±0.02^{b}	0.79±0.01^{ABb}	0.78±0.02^{Bb}	0.86±0.03^{a}
	10	0.81±0.03^{ABb}	0.76±0.03^{ABc}	0.77±0.03^{bc}	0.78±0.01^{Bbc}	0.78±0.02^{Bbc}	0.86±0.01^{a}
	15	0.85±0.03^{Aa}	0.77±0.04^{Ab}	0.73±0.06^{b}	0.74±0.01^{Cb}	0.73±0.03^{Cb}	0.86±0.02^{a}
Gumminess (kg)	0	2.63±0.15^{a}	1.46±0.21^{bc}	1.50±0.97^{bc}	1.36±0.83^{c}	1.26±0.31^{ABc}	1.85±0.52^{Ab}
	3	2.63±0.37^{a}	1.53±0.29^{b}	1.22±0.13^{b}	1.52±0.34^{b}	1.82±0.67^{Ab}	1.56±0.13^{ABb}
	7	1.94±0.29^{a}	1.35±0.30^{b}	1.23±0.22^{b}	1.33±0.16^{b}	1.48±0.20^{ABb}	1.19±0.11^{Bb}
	10	2.56±0.42^{a}	1.23±0.24^{b}	1.32±0.32^{b}	1.37±0.10^{b}	1.46±0.15^{ABb}	1.55±0.18^{ABb}
	15	2.21±0.46^{a}	1.29±0.20^{b}	1.24±0.30^{b}	1.20±0.12^{b}	1.05±0.17^{Bb}	1.47±0.08^{ABb}

[1)] The mixing ratios of the ingredients are shown in Table 1.
$^{A–C}$ Means within a column with different letters are significantly different ($p<0.05$).
$^{a–d}$ Means within a row with different letters are significantly different ($p<0.05$).

production in meat products. Mitsumoto et al. (2005) reported that catechins extracted from tea effectively suppressed lipid oxidation in beef patties and chicken patties. Ribas-Agusti et al. (2014) confirmed that sausages prepared with CEs contained polyphenolic compounds, such as catechin, procyanidin, and quercetin. Therefore, these results suggested that the antioxidant activities of polyphenolic compounds in cacao nibs effectively suppress lipid oxidation in patties during refrigerated storage.

Total microbial plate counts

As shown in Table 4, total microbial plate counts significantly increased in all groups during storage, and CEs had no significant effect on the microbial count ($p>0.05$). On day 0 of storage, the total microbial plate count was moderate, ranging

Table 4. Thiobarbituric acid reactive substances (TBARS) values and total plate counts of cooked patties formulated with different amounts of cacao nips extracts (CEs) during cold storage (4℃)

Traits	Storage periods (d)	CON	Treatment[1)]				
			CE-0.025	CE-0.05	CE-0.075	CE-0.1	ASC-0.1
TBARS (mg MDA/kg sample)	0	0.54±0.01^{Da}	0.29±0.03^{Db}	0.23±0.01^{Ec}	0.19±0.01^{DEd}	0.16±0.02^{De}	0.15±0.01^{Ee}
	3	1.21±0.01^{Ca}	0.81±0.02^{Cb}	0.50±0.01^{Dc}	0.25±0.01^{Dd}	0.22±0.00^{Cf}	0.18±0.00^{De}
	7	1.48±0.00^{Ba}	1.03±0.02^{Bb}	0.66±0.01^{Cc}	0.32±0.00^{Cd}	0.26±0.02^{Be}	0.25±0.01^{Ce}
	10	1.47±0.02^{Ba}	1.07±0.00^{Bb}	0.73±0.02^{Bc}	0.39±0.00^{Bd}	0.30±0.01^{Ae}	0.29±0.00^{Be}
	15	1.98±0.03^{Aa}	1.48±0.01^{Ab}	1.28±0.01^{Ac}	0.48±0.02^{Ad}	0.32±0.01^{Ae}	0.31±0.00^{Ae}
Total plate counts (Log CFU/g)	0	3.18±0.09^{E}	3.19±0.05^{E}	3.24±0.21^{E}	3.23±0.14^{E}	3.24±0.17^{E}	3.26±0.13^{E}
	3	3.49±0.08^{D}	3.47±0.06^{D}	3.55±0.10^{D}	3.59±0.04^{D}	3.50±0.13^{D}	3.46±0.11^{D}
	7	4.16±0.10^{C}	4.15±0.11^{C}	4.18±0.15^{C}	4.26±0.12^{C}	4.33±0.05^{C}	4.32±0.01^{C}
	10	4.51±0.12^{B}	4.50±0.08^{B}	4.46±0.09^{B}	4.43±0.09^{B}	4.38±0.09^{B}	4.46±0.16^{B}
	15	6.15±0.09^{A}	6.14±0.10^{A}	6.13±0.08^{A}	6.08±0.07^{A}	6.22±0.23^{A}	6.16±0.11^{A}

[1)] The mixing ratios of the ingredients are shown in Table 1.
$^{A–D}$ Mean within a column with different letters are significantly different ($p<0.05$).
$^{a–d}$ Mean within a row with different letters are significantly different ($p<0.05$).

from 3.18 to 3.26 Log CFU/g, and on day 15, the count ranged from 6.08 to 6.22 Log CFU/g. *T. cacao* L. has antimicrobial activity against cariogenic microorganisms, such as *Helicobacter pylori* (Lawal et al., 2014), *Streptococcus mutans*, and *Streptococcus sanguinis* (Ferrazzano et al., 2009). Several studies have reported the effects of cacao on food poisoning-related bacteria. For example, Bubonja-Sonje et al. (2011) demonstrated that the minimum inhibitory concentration of phenolic cacao extracts for *Listeria monocytogenes* is 0.4 mg/mL, and Takahashi et al. (1999) reported that cocoa extracts have antimicrobial activity against enterohemorrhagic *Escherichia coli* at a very high concentration of 8%. Therefore, the CE concentrations used in the current study were not sufficient to exhibit antimicrobial activity, and adding less than 0.1% CE would not affect the total microbial counts in pork patties. Moreover, there were no significant differences in total microbial counts between the ascorbic acid treatment and control groups during storage ($p<0.05$). Mancini et al. (2015) and Martinez et al. (2007) reported that ascorbic acid did not inhibit microbial growth on meat products. These results were consistent with previous findings that the antioxidant activities of natural antioxidants and ascorbic acid in meat products were not sufficient to suppress bacterial growth (Bannon et al., 2007; Sanchez-Escalante et al., 2001; Shivas et al., 1984).

Sensory evaluation

Sensory traits for baked pork patties prepared with CEs of different concentrations are shown in Table 5. Color preference was the highest for samples prepared using 0.075% CE (score: 5.60). Samples prepared using 0.1% ascorbic acid had the lowest flavor score, and flavor scores tended to increase with increasing CE content ($p<0.05$). As mentioned above, during the roasting of cacao nibs, melanoidin is formed, which produces the unique flavor of cacao products (Oliviero et al., 2009), which improved flavor preference. For this reason, the flavor score of ASC is considered to be relatively low. There were no significant differences in tenderness, and juiciness preference was the highest for samples prepared using 0.05% CE (score: 6.30; $p<0.05$). In terms of overall acceptability, control samples and samples prepared using 0.05% and 0.075%

Table 5. Sensory evaluation of the cooked patties formulated with different amounts of cacao nips extracts (CEs)

Traits	CON	Treatment[1)]				
		CE-0.025	CE-0.05	CE-0.075	CE-0.1	ASC-0.1
Color	5.25±0.43^{ab}	4.30±0.42^{abc}	4.45±0.47^{abc}	5.60±0.42^{a}	3.70±0.34^{bc}	3.45±0.24^{c}
Flavor	3.70±0.29^{bc}	3.95±0.32^{bc}	4.70±0.52^{ab}	5.70±0.40^{a}	5.60±0.31^{a}	2.95±0.45^{c}
Tenderness	5.30±0.55^{a}	5.60±0.34^{a}	5.50±0.43^{a}	5.15±0.46^{a}	3.90±0.27^{a}	3.95±0.45^{a}
Juiciness	4.70±0.52^{bc}	4.20±0.34^{bc}	6.30±0.19^{a}	5.60±0.42^{ab}	4.30±0.42^{bc}	3.65±0.30^{c}
Overall acceptability	6.05±1.43^{a}	4.85±1.84^{b}	6.80±1.60^{a}	6.15±1.63^{a}	4.75±1.48^{b}	3.75±2.51^{b}

[1)] The mixing ratios of the ingredients are shown in Table 1.
[a–c] Mean within a row with different letters are significantly different ($p<0.05$).

CEs had the highest scores; samples prepared using 0.05% CEs had a slightly, but insignificantly higher mean value than control samples ($p>0.05$). In line with our findings, Ribas-Agusti et al. (2014) reported that cacao extract enhanced the overall sensory quality of pork sausages, and Lise et al. (2004) reported that coffee extracts enhanced the flavor preference in pork patties. Therefore, the characteristic color and flavor of cacao nibs enhanced the quality characteristics of pork patties, and adding 0.05% or 0.075% CE improves the sensory traits of pork patties.

Conclusions

We investigated the effects of CEs on suppressing lipid oxidation and enhancing the quality traits of pork patties. CEs prepared using 70% ethanol had the highest TPC and TFC contents and the highest DPPH and $ABTS^{+}$ radical scavenging activities. In experiments using CEs- and ascorbic acid-treated pork patties, CEs had positive effects on redness discoloration and texture deterioration of pork patties during storage. Additionally, all CEs treatments suppressed fat oxidation in pork patties, and 0.1% CEs suppressed fat oxidation to a level similar to that of 0.1% ascorbic acid. CEs at concentrations of less than 0.1% did not affect the total microbial counts in pork patties. In sensory evaluations, flavor score for pork patties differed according to CEs concentrations, and overall acceptability was the highest in the Control and 0.05% CE and 0.075% CE-containing samples. Considering the sensory evaluation results and the changes in quality characteristics throughout the storage period, 0.05% CE and 0.075% CE improved consumer preference compared to ascorbic acid, an antioxidant used in commercial meat products. Therefore, we conclude that 70% ethanol solution is optimal for extracting flavonoids from cacao nibs, and that 0.05% or 0.075% CEs can be added to pork patties to produce high-quality products from a commercial point of view. In addition, CEs can improve physicochemical and sensory quality characteristics of meat products. Further research is needed to improve the quality characteristics of various meat products.

Conflict of Interest

The authors declare no potential conflict of interest.

Author Contributions

Conceptualization: Choi HY, Kim GW. Data curation: Choi JH. Formal analysis: Kim NM. Methodology: Choi HY, Kim GW. Software: Choi JH. Validation: Choi JH, Kim NM. Investigation: Choi JH, Kim NM. Writing - original draft: Choi JH.

Writing - review & editing: Choi JH, Kim N, Kim GW, Choi HY.

Ethics Approval

The study was approved by the Institutional Review Board of Kongju National University (approval number: KNU_IRB_2018-74) on December 11, 2018.

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