Korean Journal for Food Science of Animal Resources

Korean J. Food Sci. An. 2018 October 38(5):889~900
DOI https://doi.org/10.5851/kosfa.2018.e22

pISSN : 1225-8563 eISSN : 2234-246X
www.kosfa.or.kr

ARTICLE

Evaluation of *Cudrania tricuspidata* Leaves on Antioxidant Activities and Physicochemical Properties of Pork Patties

Tran Van Cuong[1,2] and Koo Bok Chin[1,*]

[1]Department of Animal Science, Chonnam National University, Gwangju 61186, Korea
[2]Department of Food Science and Biotechnology, Tay Nguyen University, 567 Le Duan, Dak Lak, Vietnam

OPEN ACCESS

Received April 24, 2018
Revised June 15, 2018
Accepted July 25, 2018

***Corresponding author** : Koo Bok Chin
Department of Animal Science, Chonnam National University, Gwangju 61186, Korea
Tel: +82-62-530-2121
Fax: +82-62-530-2129
E-mail: kbchin@chonnam.ac.kr

Abstract Characterization and utilization of the bioactive compounds from natural resources is one of the most concerns to maintain quality properties of foods, especially to prevent the oxidation of lipids in meat products. Phytochemical components and antioxidant activities of *Cudrania tricuspidata* (CT) leaves extracted using various solvents and their effects on physicochemical properties of pork patties during refrigerated storage were measured. The combined solvents of 80% ethanol, 80% methanol and pure double-distilled (dd)-water obtained the higher total phenolic compounds, flavonoids content, and antioxidant activities as compared to the pure solvent alone. Among the individual antioxidant components, catechin was the predominant polyphenol in CT leaves in all extracts. The addition of CT leaves extracts into pork patties showed high antioxidant activities since thiobarbituric acid-reactive substances (TBARS) values of added CT extracts were lower than those of the control ($p<0.05$). In conclusion, CT leaf phytochemical components displayed antioxidant activity that varied with the extract solvent used. CT extracts were superior to control in retarding lipid oxidation of pork patties, which was evident as reduced TBARS and peroxide values (POV).

Keywords antioxidant activity, *Cudrania tricuspidata*, lipid oxidation, phytochemical components, pork patties

Introduction

Phytochemical components, especially polyphenol compounds, are secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways. They have potent antioxidant activities *in vitro* due to their high reactivity as hydrogen or electron donors, and also chelate metal ions and scavenge free radicals (Meot-Duros and Magné, 2009). Phenolic compounds have been associated with physiologic health benefits associated with their anti-allergenic, anti-atherogenic, antiinflammatory, antimicrobial, antioxidant, anticarcinogenic, antimutagenic, antithrombotic, cardioprotective, and vasodilatory effects (Kchaou et al., 2013). Pork

meat is a popular food globally. As with the other foods, ensuring the quality and safety of pork meat is of paramount importance. Oxidation of lipids and proteins is a main cause of meat deterioration and economic loss in the meat industry. Synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate (PG), are used to prevent lipid oxidation with the goals of minimizing formation of toxic oxidation products, maintaining nutrition and sensory quality, and extending the shelf-life of muscle-based foods like pork meat during storage However, it is still unclear whether chronic consumption of synthetic antioxidants poses health risks. Interest is growing in the potential value of natural antioxidants in the food production sector, with phenolic compounds being of particular interest.

Cudrania tricuspidata (CT, also known as silkworm thorn and store-house-bush) is a deciduous tree and a member of the Moraceae family that grows naturally in Asian countries including Korea, China, and Japan (Kim et al., 2015). It has been used as a traditional medicine for a long time. CT leaves possess antioxidant, anti-inflammatory, antitumor, and α-glucosidase inhibition activities (Kim et al., 2015). Total phenolic compounds and flavonoids of aqueous extracts from the fruit, root, leaf, and stem of CT have been studied concerning the protection of neuronal cells from oxidative stress-induced cytotoxicity (Jeong et al., 2010).

The effects of various solvents used in the extraction of CT components on phytochemical and antioxidant activities are unknown. Furthermore, the potential value of CT extracts in meat production has not been reported. The present study evaluated the effectiveness of CT leaf extracts on antioxidant activities and phytochemical components, and their effects on physicochemical properties of pork patties during refrigerated storage.

Materials and Methods

Experiment I: Effects of solvents on CT leaf antioxidant activities and phytochemicals

Preparation of CT leaf extracts

Fresh CT leaves were obtained from a local market in Gwangju, Korea. The leaves were oven-dried at 50℃ until the weight was constant. After drying, they were well ground with Ultra-Power mixer (Hanil, Korea) operating at 650 W. The ground material was passed through a testing sieve with a 200 μm pore size (Chung Gye Sang Gong Sa, Korea). The powder was extracted using five different solvents (ethanol 80% (EtOH80), pure ethanol (EtOH100), methanol 80% (MeOH80), pure methanol (MeOH100), and double-distilled (dd)-water; the ratio of powder to solvents=1:100) with a magnetic stirrer for 12 hs at room temperature. The mixture was then centrifuged at 3,000 rpm for 5 min (VS-5000N, Vision scientific Co. Ltd., Korea). Once centrifuged, the mixtures of solid-liquid were filtered using Whatman #1 filter paper. The ethanol/methanol was removed from the extracted solution using a rotary evaporator (R-100 Rotary Evaporator, Switzerland). Thereafter, the extract was held at –70℃ prior to lyophilizing at –55℃ (Ilshin Freeze Dryer, Korea) until completely dry for about 4 days. All samples were then kept in a refrigerator at 2℃ before analyzing the phytochemical compounds, antioxidant activity, and the manufacture of pork patties.

Antioxidant analyses of CT leaf extracts

Total phenolic compounds (TPC) and ascorbic acid (AA) content

TPC was measured as gallic acid equivalents (GAE) using the Folin–Ciocalteau's phenol reagent (FC reagent) as previously described (Lin and Tang, 2007). AA content was determined as previously described by comparison to a series of AA standard solutions (0–200 μg/mL) (Park et al., 2008).

Total flavonoids

Total flavonoids were determined using a previously described aluminum chloride colorimetric method with slight modification (Lin and Tang, 2007). The absorbance of the mixture was measured at 415 nm using a model UV 1601 ultraviolet-visible (UV-Vis) spectrophotometer (Shimadzu, Japan). Total flavonoids were calculated and expressed as mg quercetin equivalent per g of dried leaf powder.

Antioxidant activity

In vitro antioxidant assays of the CT extracts included 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, ferrous ion chelating activity, reducing power, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid, ABTS) radical scavenging decolorization activity, and total antioxidant capacity. All have been previously described and were slightly modified (Cuong and Chin, 2016).

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

HPLC analysis of major phenolic and flavonoid components from CT extracts utilized the LC-10Avp HPLC system (Shimadzu, Japan) equipped with a Shim-pack CLC-ODS C18 column (Shimadzu, Japan). The absorbance was read using the aforementioned UV-Vis spectrophotometer at a wavelength of 280 nm. Identification of the phenolic and flavonoid compounds was done by comparing their retention times to those of gallic acid, catechin, vanillic acid, rutin, and quercetin standards.

Experiment II: Effects of CT leaf extracts on physicochemical properties of pork patties during refrigerated storage

Preparation of extracts for manufacture of pork patties

Based on the results of the phytochemical analysis and antioxidant activities of CT extracts using the various solvents, the three most effective solvents were used (dd-water, 80% ethanol [EtOH], and 80% methanol [MeOH]). The extracts were evaporated using a rotary vacuum evaporator prior to freeze drying at –55°C in a commercial freeze drier (Ilshin, Korea) until completely dried (about 5 d).

Processing of pork patties

Pork patties were prepared using 1.0 g of dd-water, EtOH80, or MeOH80 CT extract per 100 g of patties (TRT1-3), as well as the same concentration of control (CTL) (no addition of extract) and a reference (REF; the addition of AA). Patties with an average diameter of 8.5 cm diameter and thickness of 1 cm were formed using a conventional patty-maker. The patties were refrigerated for 14 days at 4±1°C.

Physicochemical and antioxidative activities of pork patties

pH and color values

pH values were measured using a MP-120 pH meter (Mettler-Toledo, Switzerland). Color values of patties were determined using a CR-10 color reader (Minolta Co. Ltd., Japan) and reported as lightness (L*), redness (a*), and yellowness (b*). A numerical total color difference (ΔE) between patties at storage day 0 and 14 was calculated as:

$$\Delta E_{0-14}=[(L_{14}-L_0)^2+(a_{14}-a_0)^2+(b_{14}-b_0)^2)]^{1/2}$$

Thiobarbituric acid reactive substances (TBARS)

Secondary products of lipid oxidation during storage were determined as TBARS as previously described (Sinnhuber and Yu, 1977). The reactive substances were measured at 532 nm using the aforementioned UV-1601 spectrophotometer. TBARS was calculated as:

TBARS (mg malondialdehyde [MDA]/kg)=optical density [Absorbance]×9.48/sample weight (g).

Peroxide value (POV)

POV was determined using a previously described spectrophotometric method with modification (Shantha and Decker, 1994). The absorbance of the sample was measured at 500 nm against a blank that contained all the reagents except the samples using the aforementioned spectrophotometer, with results expressed in milliequivalents of oxygen per kilogram of pork patty (meq/kg).

Statistical analyses

All experiments were carried out in triplicate, data were analyzed, and statistical comparisons were made by using one-way and two-way analysis of variance (ANOVA). Significant differences were assessed by the Duncan post hoc test at p-value <0.05 using Statistical Package for the Social Sciences version 20.0 for Windows software (SPSS Inc., USA).

Results and Discussion

Experiment I: Effects of solvents on antioxidant activities and phytochemicals from CT leaf

Bioactive compounds

As shown in Table 1, TPC of CT leaf extracts ranged from 37.64 to 58.66 mg GAE/g, which was highest in the EtOH80 extract followed by MeOH80 and pure dd-water, with significantly less TPC obtained in the pure ethanol and pure methanol extracts ($p<0.05$). These results disagreed with earlier observations, that dd-water was a less efficient solvent for the extraction of plant TPC than the other solvents (Wijekoon et al., 2011). However, our results agreed with previous researchers, who reported that water extracts contained the highest polyphenol contents from papaya leaf and black tea, as compared to pure acetone, EtOH, and MeOH (Vuong et al., 2013). These results also indicated the variation in the type and quantity of phenolic compounds extracted by different solvents. In previous studies, TPC extraction was affected by the change of solvent polarity, extraction conditions (vapor pressure, ratio, time extraction, temperature) and viscosity (Fernández-Agulló et al., 2013). In general, a direct relationship was evident between the amount of extracted phenolic compounds and solvent polarity. Low-viscosity solvents have low density and high diffusivity, which allows them to readily diffuse into the pores of the plant matrices to leach out the solvent-soluble bioactive components (Wijekoon et al., 2011). In this study, the aqueous 80% EtOH and MeOH solvents extracted more TPC than pure EtOH and MeOH (both $p<0.05$). Aqueous mixtures of organic solvents have been reported to be more efficient in extracting phenolic compounds than pure solvents (Fernández-Agulló et al., 2013). However, Thoo et al. (2010) reported that more TPC was extracted using lower EtOH concentrations, which could reflect the sensitivity of leaf TPC and their differing extraction by solvents of different polarity. Plants contain diverse

Table 1. Effects of extracting solvents on the phytochemical compositions (TPC, TFC, AA) and total antioxidant capacity (TAC) from *Cudrania tricuspidata* leaf

Solvents	TPC[1)]	TFC[2)]	AA[3)]	TAC[4)]
EtOH				
80%	58.66±3.22[a]	10.07±0.56[a]	9.50±0.33[a]	26.58±0.84[b]
pure	37.64±2.71[c]	3.08±0.20[d]	6.23±0.76[b]	18.64±0.57[d]
MeOH				
80%	53.47±4.04[ab]	8.40±0.53[b]	8.90±1.13[a]	28.23±0.76[a]
pure	39.37±2.32[c]	4.69±0.18[c]	6.05±0.75[b]	22.60±0.42[c]
dd-water	48.88±3.31[b]	7.59±0.76[b]	6.01±0.77[b]	28.71±1.36[a]

[a–d] Values (mean±SD, n=3), different superscript letters in a same column indicate significant differences at $p<0.05$.
[1)] Total phenolic compound: mg gallic acid equivalent (GAE)/g d.m. of leaves powder.
[2)] Total flavonoids content: mg quercetin equivalent (QE)/g d.m. of leaves powder.
[3)] Ascorbic acid content: mg (AA)/g d.m. of leaves powder.
[4)] Total antioxidant capacity: mg ascorbic acid equivalent in (AAE)/g d.m. of leaves powder.
EtOH, ethanol; MeOH, methanol; dd-water, double, distilled water.

phenolic compounds that display antioxidant activity, but differ in polarity. TPC extraction will change depending on the polarity of the solvent used.

CT leaf total flavnoids content (TFC) varied from 3.08 to 10.07 mg QE/g and was different among the extracts, being highest in EtOH80 followed by MeOH80, dd-water, MeOH100, and EtOH100 ($p<0.05$). These results clearly demonstrated that combined solvents were more appropriate for extraction of TFC than the pure solvents. Thus, pure dd-water might be a proper extraction solvent, especially considering its non-toxic and chemical-free nature.

AA contents in the EtOH80 and MeOH80 extracts were higher than those in dd-water, pure EtOH, and pure MeOH ($p<0.05$; Table 1). Interestingly, EtOH80 and MeOH80 were more efficient for AA extraction than dd-water, even though AA was water soluble. A mixture of water and organic solvents might provide better contact and interactions of solvents in and out of the leaf powder, as compared to the pure solvents.

TAC of different solvent extracts was measured and expressed as mg ascorbic acid equivalents (AAE)/g dried leaves using the phospho-molybdenum method. DD-water and MeOH80 had the highest antioxidant activity (28.71 and 28.23 mg AAE/g, respectively), followed by EtOH80 (26.58 mg AAE/g) and pure MeOH (22.60 mg AAE/g), whereas pure ethanol at 18.64 mg/g was the lowest among extracts ($p<0.05$; Table 1). These differences in TAC indicated that change in polarity and vapor pressure of the solvent might influence the antioxidant activity. Extraction conditions and procedures might also affect the antioxidant activity (Zhou and Yu, 2004). Previous studies measured the effect of different solvents on antioxidant activity using different methods and reported that 70% MeOH extract exhibited strong antioxidant activity (Sahreen et al., 2010), while, Do et al. (2014) reported that 100% EtOH extract had the highest total antioxidant activity, followed by 100% acetone and 100% MeOH. Presently, dd-water extracts had strong TAC, since most antioxidant compounds in CT leaf are water-soluble.

Antioxidant activities

DPPH radical scavenging activity of the five water or alcohol extracts are presented in Table 2. MeOH extract contained substantially higher DPPH than EtOH extract. DPPH of MeOH and EtOH extracts ranged from 86.3% to 89.5% and 81.4% to 86.2%, respectively, at concentrations from 1–10 mg/mL. DD-water extract showed the lowest activity, from 32.3% to 68.9%,

Table 2. ***In vitro*** **antioxidant assays of** ***Cudrania tricuspidata*** **leaf by various solvents at different concentration**

Parameters	Treatments	Concentration (mg/mL)				
		0	1.0	2.5	5.0	10.0
DPPH radical scavenging (%)	EtOH80	0^{aC}	82.7^{aB}	84.7^{bAB}	85.3^{aA}	85.2^{bA}
	EtOH100	0^{aD}	81.4^{aC}	83.3^{bBC}	84.4^{aAB}	86.2^{bA}
	MeOH80	0^{aB}	87.5^{aA}	88.4^{abA}	89.5^{aA}	87.3^{abA}
	MeOH100	0^{aB}	87.1^{aA}	86.3^{abA}	86.9^{aA}	89.2^{abA}
	dd-Water	0^{aD}	32.4^{bC}	45.5^{cBC}	50.6^{bB}	68.9^{cA}
	AA	0^{aB}	86.2^{aA}	93.5^{aA}	92.9^{aA}	93.7^{aA}
Ferrous ion chelating (%)	EtOH80	0^{aE}	23.5^{cD}	47.2^{bC}	89.5^{bB}	101.2^{abA}
	EtOH100	0^{aE}	10.2^{dD}	23.2^{dC}	33.0^{dB}	39.1^{dA}
	MeOH80	0^{aE}	15.7^{dD}	46.3^{bC}	93.9^{abB}	100.5^{bA}
	MeOH100	0^{aD}	14.3^{dC}	30.6^{cB}	43.6^{cA}	44.2^{cA}
	dd-Water	0^{aD}	84.7^{bC}	95.6^{aB}	98.9^{aB}	104.5^{aA}
	EDTA	0^{aB}	99.9^{aA}	99.9^{aA}	100.1^{aA}	100.8^{abA}
Reducing power (Abs700)	EtOH80	0.22^{aE}	0.63^{bD}	1.25^{bC}	2.14^{bB}	2.68^{abA}
	EtOH100	0.22^{aE}	0.40^{cD}	0.69^{cC}	1.15^{cB}	2.01^{dA}
	MeOH80	0.22^{aE}	0.64^{bD}	1.26^{bC}	2.15^{bB}	2.55^{bA}
	MeOH100	0.23^{aE}	0.43^{cD}	0.79^{cC}	1.35^{cB}	2.20^{cA}
	dd-Water	0.21^{aE}	0.57^{bD}	1.12^{bC}	1.97^{bB}	2.63^{bA}
	AA	0.22^{aD}	2.36^{aC}	2.43^{aC}	2.54^{aB}	2.80^{aA}
ABTS radical scavenging (%)	EtOH80	0^{aE}	13.4^{aD}	28.1^{aC}	47.3^{aB}	78.2^{aA}
	EtOH100	0^{aE}	6.8^{bD}	16.3^{cC}	30.6^{dB}	54.8^{cA}
	MeOH80	0^{aE}	12.1^{aD}	23.8^{bC}	43.2^{bB}	73.1^{bA}
	MeOH100	0^{aE}	7.7^{bD}	16.2^{cC}	35.0^{cB}	55.1^{cA}
	dd-Water	0^{aE}	5.8^{bD}	13.3^{dC}	25.3^{eB}	53.7^{cA}

$^{a–e}$ Means with different superscript letters in the same column indicate significant differences at $p<0.05$.
$^{A–E}$ Means with different superscript letters in the same row indicate significant differences at $p<0.05$.
EtOH, ethanol; MeOH, methanol; dd-water, double, distilled water; AA, ascorbic acid; EDTA, ethylenediamine-tetra acetic acid disodium salt dihydrate.

while AA, as a positive reference had an activity ranging from 86.2% to 93.7%. These findings agreed with previous results that extracts obtained using pure and aqueous organic solvents displayed stronger DPPH radical scavenging ability than water extract (Do et al., 2014). Thus, lower polarity solvents tended to be markedly more effective in extracting of radical scavenging plant compounds. Bioactive compounds in the leaf contain both simple and complex groups that differ in polarity. According to Zhou and Yu (2004), changes in solvent polarity altered its ability to dissolve a selected group of antioxidant compounds and influenced the antioxidant activity. In this study, solvents at different concentrations resulted in extracts having similar activity to AA, except for dd-water extract. The radical scavenging ability of MeOH and EtOH extracts was independent of solvent concentration. These results were supported by previous findings of similar DPPH radical scavenging activity of extracts obtained using various organic solvents at different concentrations.

The results of the ferrous ion chelating test plotted as percentage chelating as affected by the various solvent extracts are

shown in Table 2. The positive standard (EDTA; 1–10 mg/mL) showed very strong ferrous ion chelating activity of 99.88%–100.8%. The ferrous ion chelating activity of the various extracts in descending order was dd-water>EtOH80≈MeOH80> MeOH100>EtOH100. EtOH80 and MEOH80 extracts had higher activity than their corresponding pure solvents ($p<0.01$; Table 2), while the dd-water extract of CT leaves showed strong ferrous ion chelating activity (84.7%–104.5%) that was similar to the reference (EDTA).

Therefore, TPC and TFC from CT leave chelate ferrous iron by forming complexes with metal ions. Pure dd-water extracts had higher chelating activity than MeOH80 and EtOH80, whereas pure MeOH and EtOH showed the lowest activities ($p<0.05$). These might be due to the complex composition of CT leaves, which contained compounds with various antioxidant potential that differ in polarity and mechanisms with a higher proportion of hydrophilic compounds. In this case, dd-water was a more effective solvent to extract ion chelating compounds in the leaf, as compared to the other solvents. The result is similar to the observation of Yesiloglu and Sit (2012), who reported a higher ion chelating capacity of 100 μg/mL in water than in EtOH or acetone. These results suggested that pure water extract might be a better solvent for extraction of ion chelating compounds from the CT leaf. In a previous study, the differences in the structure of phenolic components, as well as the methodology of the antioxidant assay, might have been the basis of the different results in the assessment of antioxidant ability (Celep et al., 2012). Therefore, the analysis of antioxidant activities from plant extracts must use various *in vitro* assays for different mechanisms to obtain meaningful results.

The reducing power of various extracts obtained using solvents at different concentrations is presented in Table 2. The extraction solvents affected the reducing power ($p<0.05$). The absorbance increased linearly with increasing concentration of the extracts. Reducing power was dependent on the different types and grades of solvent used (Table 2). Higher reducing power was obtained using EtOH80, MeOH80, and dd-water as compared to the pure EtOH and MeOH. Our results differ from the finding of Anwar and Przybylski (2012), who reported that reducing power was highest in 100% MeOH extract, exceeding the present values of the MeOH80 and EtOH80 extracts. The differences between these two results could be partially due to the variety of the plant materials obtained by various mechanisms, which might contribute to the oxidative processes. The higher reducing power in an aqueous solvent at 80% and pure water rather than pure ethanol and methanol could be attributed to the differences in the polarity, vapor pressure, and viscosity of each solvent. Data concerning the ability of the various extracts to donate hydrogen in scavenging $ABTS^+$ are shown in Table 2. Activity was the highest in EtOH80 extract, followed by the MeOH80 extract; the activity of extracts obtained from pure EtOH, pure MeOH, and dd-water were similar or lower. A linear correlation between the concentration of the various extracts (0–10 mg/mL) and discoloration of the $ABTS^+$ solution were observed. This means that the radical-scavenging ability increased with increasing concentration of all extracts.

Individual antioxidant components from CT leaf extracts

Since EtOH80, MeOH80, and dd-water were the effective solvents in terms of bioactive compounds and antioxidant activity in this study, they were further analyzed by HPLC. Comparison with individual antioxidant components including vanillic acid, quercetin, rutin, gallic acid and catechin, allowed the extracted compounds to be detected and quantified by HPLC. Five individual antioxidant components were identified in all three extracts (Table 3). Catechin was the predominant polyphenol in CT leaf followed by quercetin. The quercetin contents of the EtOH80 and MeOH80 extracts were significantly higher than those in the dd-water extract ($p<0.05$), perhaps reflecting the low water solubility of quercetin. In contrast, gallic acid and rutin contents of the dd-water extract were markedly higher than those of the EtOH80 extract. The HPLC results

Table 3. **Individual antioxidant components of *Cudrania tricuspidata* leaf by different extraction methods**

Treatments	Individual antioxidant components[1)]				
	Gallic acid	Catechin	Vanillic acid	Rutin	Quercetin
EtOH80	67.57[b]	6,418.31[a]	76.45[a]	53.55[c]	1,126.15[a]
MeOH80	124.11[a]	8,258.43[a]	85.28[a]	212.57[a]	971.05[b]
dd-Water	120.25[a]	4,633.54[a]	65.49[a]	97.26[b]	629.41[c]

[a-c] Mean with different superscript letters in the same column indicate significant differences at $p<0.05$.
[1)] mg/100 g extracted d.m. leaves powder.

were consistent with the TPC and total flavonoid contents, confirming the different bioactive profiles of CT leaf extracts using the different solvents. These results also supported that dd-water was a good candidate for extracting the main bioactive components from CT. Gallic acid, catechin, rutin, and quercetin could be major antioxidant components in this leaf. Flavonoids and phenolic acids are also major compounds that contribute to the antioxidant activity of natural antioxidant resources and which have health benefits. These results indicated that CT leaf could be a potential source of antioxidants.

Experiment II: Effects of various solvent extracts from CT leaves on the physicochemical properties of pork patties during refrigerated storage

pH and Hunter color values

The changes of pH of pork patties made by incorporation of the various CT leaf solvent extracts during storage are shown in Table 4. The pH ranged from 5.76 to 5.86, with no difference among different types of patties ($p>0.05$), indicating that CT extracts did not affect the pH of pork patties. pH increased from 5.74 to 5.83 at day 3, decreased to 5.76 at day 7 and rose to 5.90, thereafter (Table 4). These results were supported by previous findings of increased pH during refrigerated storage of meat products (Li et al., 2014). These results might be partially due to the production of alkaline substances, such as ammonia and amine groups (Li et al., 2014). A previous study reported that the increasing of pH values of fresh pork during refrigerated storage was mainly due to the breakdown of protein and subsequent formation of amino acids produced by bacteria in the meat (Miao et al., 2015).

Color values of pork patties during storage are presented in Table 4. The addition of CT extracts had a significant effect on the color displayed by freshly made patties. Lightness (L*) and redness (a*) values of mixed CT solvent extract were lower than those of control or reference samples ($p<0.05$). CT extracts had an intense greenish color as a result of chlorophylls compound, which strongly affected to the surface color of patties containing CT. Several studies reported a correlation between plant extracts and surface colors of meat products (Kim et al., 2013). The redness (a*) of the control and reference samples decreased more than those of the other treatments, which might be due to the denaturation of myoglobin or the oxidation leading to iron oxidation (Kim et al., 2013). Control patties were lighter than references patties or patties containing CT extract ($p<0.05$). The nature of the extract made no difference to the L* values. Redness (a*) values also significantly decreased in all the treatments during storage time ($p<0.05$), however the decrease was non-significant in the first 7 days ($p>0.05$); but increased thereafter ($p<0.05$). Rodríguez-Carpena et al. (2011) reported that the addition of avocado by-products (seeds and peels) extracts maintained the color stability of raw porcine patties during storage as compared to control samples. The similarity was also observed by Shan et al. (2009), who stated that spice and herb extracts could retard color deterioration during storage of raw pork meat.

Table 4. Physicochemical properties of pork patties with addition *Cudrania tricuspidata* leaf by various extracts

Variable	Parameters[1)]					
	pH	L*	a*	b*	TBARS	POV
TRT storage	NS	NS	NS	NS	***	NS
Treatment[2)]	NS	***	***	***	***	***
Storage	*	NS	*	NS	***	*
Treatment						
CTL	5.81	60.12^{a}	8.37^{b}	9.08^{d}	1.53^{a}	7.27^{a}
REF	5.86	57.39^{b}	11.11^{a}	9.38^{cd}	0.21^{b}	1.69^{c}
TRT1	5.80	51.62^{c}	3.22^{d}	12.71^{b}	0.21^{b}	7.37^{a}
TRT2	5.81	49.92^{c}	1.56^{e}	14.21^{a}	0.25^{b}	8.38^{a}
TRT3	5.76	52.35^{c}	5.56^{c}	10.36^{c}	0.22^{b}	3.52^{b}
Storage time (days)						
0	5.74^{c}	52.97	7.10^{a}	11.47^{a}	0.31^{d}	5.04^{b}
3	5.83^{ab}	53.25	7.17^{a}	10.60^{a}	0.39^{cd}	5.31^{b}
7	5.76^{b}	54.66	6.31^{a}	11.04^{a}	0.48^{bc}	5.03^{b}
10	5.82^{ab}	55.04	4.76^{b}	11.10^{a}	0.58^{ab}	6.89^{a}
14	5.90^{a}	55.49	4.47^{b}	11.53^{a}	0.68^{a}	5.96^{ab}

$^{a–d}$ Means with different superscript letters in a same column indicate significant differences at $p<0.05$.
[1)] Parameters: L, lightness; a, redness; b, yellowness; TBARS, thiobarbituric acid reactive substances (mg MDA/kg); POV, peroxides value (meq/kg).
[2)] Treatment: CTL, patties without addition; REF, 0.1 g/100 g of ascorbic acid; TRT1, 1.0 g/ 100 g leaves powder by methanol 80% extraction; TRT2, 1.0 g/ 100 g leaves powder by ethanol 80% extraction; TRT3, 1.0 g/ 100 g leaves powder by dd-water extraction.
* $p<0.05$, *** $p<0.001$.
NS, not significant.

Lipid oxidation during storage

As shown in Table 4, TBARS of control patties were higher than those of the other treatments ($p<0.05$), but was not different among patties containing CT extracts or AA (Fig. 1) ($p>0.05$). TBARS in pork patties containing CT leaf extracted using EtOH80, MeOH80, and pure dd-water were similar values to those of reference (AA) patties during storage up to 14 days. The high level of TPC from CT leaf in all three extracts led to the lowering of TBARS by preventing lipid oxidation in the patties. The positive effect of phenolic compounds from natural resources on lipid oxidation has been fully demonstrated. Phenolic compounds and the other biochemical compounds from plant pigment mainly contributed to the antioxidant activities, as well. They donate hydrogen atoms and inhibit free radical formation and the propagation of free radical reactions through the chelation of transition metal ions, particularly iron and copper (Kim et al., 2013). The TBARS values significantly increased in day 7 in the raw patties, indicating that the patty samples underwent lipid oxidation. Especially, TBARS was higher than 1.5 mg MDA/kg meat at day 7 in control samples (Fig. 1), which was considered as an unacceptable value in raw meats. This agrees with the findings of several authors, who reported much higher TBARS values in control pork patties than those in patties containing added natural extracts (Kim et al., 2013). These results indicated that lipid oxidation was partially retarded by antioxidants from various CT leaf extracts during refrigerated storage of pork patties.

As shown in Table 4, POV of reference patties were lowest, followed by pork patties containing pure dd-water extract, with similar or significantly higher values evident for patties formed with an extract obtained using EtOH80 and MeOH80, and in control samples (all $p<0.05$). Higher levels of POVs in control and TRT1 and TRT2 samples, as compared to the reference

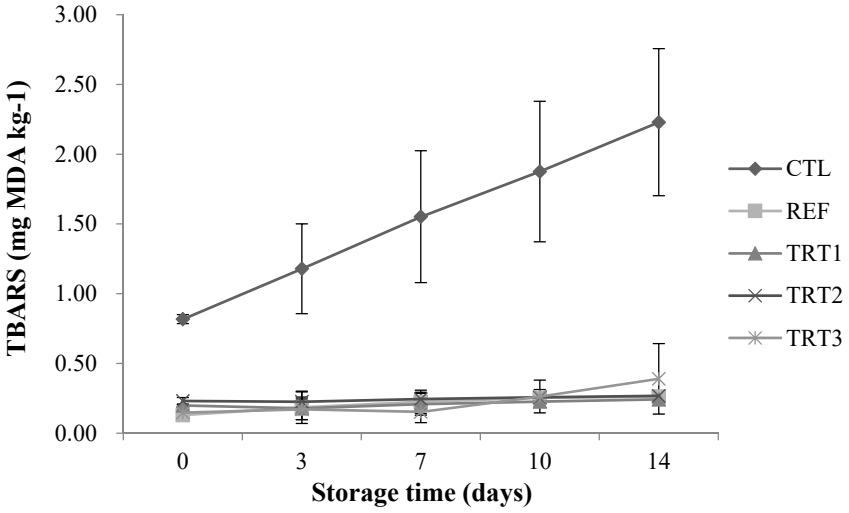

Fig. 1. Changes in TBARS values during storage of pork patties by different treatments. TBARS, thiobarbituric acid-reactive substances, Treatment: CTL, patties without addition; REF, 0.1 g/100 g of ascorbic acid; TRT1, 1.0 g/ 100 g leaves powder by methanol 80% extraction; TRT2, 1.0 g/ 100 g leaves powder by ethanol 80% extraction; TRT3, 1.0 g/ 100 g leaves powder by dd-water extraction.

and TRT3, were partially due to the decomposition of unsaturated lipid, resulting in the formation of hydroperoxides. In patties formed using CT leaf extracts, dd-water extract yielded the lowest POV among the treatments and control during storage. Thus, treatments of pork patties with AA (reference) and CT leaf extracted using dd-water was more efficient in lowering POV than those in MeOH80 and EtOH80 solvents, and control samples. POVs rapidly increased after day 7, had the highest value at day 10 ($p<0.05$), and then decreased thereafter (Table 4). The decreases in POV after day 10 could be highly related to the mechanism of lipid oxidation. Hydroperoxide compounds, which are the primary product of lipid oxidation, were detected by POV determination methods; these primary products are unstable and easy to degrade and form secondary products, which cannot be detected by POV assay.

Conclusion

EtOH and MeOH applied at 80% yield higher total phenolic compounds, flavonoids, and antioxidant activities than the pure solvents. The addition of 1.0 g/100 g CT leaf powder prepared following extraction with 80% MeOH, EtOH, and dd-water to pork patties was effective in maintaining color and preventing lipid oxidation. Although CT leaf could be considered a good source of antioxidant with 80% EtOH and MeOH, and dd-water, pure dd-water extracts from CT leaf could be potential as an ingredient for the production of pork patties with improved antioxidant activity and safer for the consumers.

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