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Antioxidant Properties of Teaw (*Cratoxylum formosum* Dyer) Extract in Soybean Oil and Emulsions

PITCHAON MAISUTHISAKUL,† RUNGNAPHAR PONGSAWATMANIT,† AND MICHAEL H. GORDON*,‡

Department of Product Development, Faculty of Agro-Industry, Kasetsart University, 50 Phaholyothin Rd., Chatuchak, Bangkok 10900, Thailand, and Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, The University of Reading, Whiteknights P.O. Box 226, Reading RG6 6AP, United Kingdom

The antioxidant activity of an extract from Teaw (*Cratoxylum formosum* Dyer) leaves was studied in soybean oil and soybean oil-in-water emulsions. Samples containing the extract or reference antioxidants including chlorogenic acid, which comprises 60% of the Teaw extract, were stored at 60 °C and analyzed periodically for peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) to allow both hydroperoxides and hydroperoxide degradation products to be monitored. Chlorogenic acid and the Teaw extract were more effective than α-tocopherol in inhibiting lipid oxidation in bulk oil but were less effective in an oil-in-water emulsion in accordance with the polar paradox. The PV/TBARS ratio for oil samples containing chlorogenic acid was higher than for α-tocopherol and BHT because chlorogenic acid inhibits both hydroperoxide formation by radical scavenging and hydroperoxide decomposition by metal chelation. The importance of the metal-chelating activity in retarding hydroperoxide decomposition was confirmed by studying the decomposition of oil samples containing added ferric ions. The PV/TBARS ratio was higher for citric acid than for α-tocopherol in the presence of added ferric chloride, but the order was reversed in samples lacking ferric chloride. Samples containing added chlorogenic acid gave the highest PV/TBARS ratios both in the presence and absence of ferric ions. The PV/TBARS ratios for the samples containing antioxidants fell rapidly to lower values in a soybean oil-in-water emulsion than in the soybean oil. This was due to increased hydroperoxide decomposition in the emulsion at the same PV. The Teaw extract contained 12% oil-soluble components, which contributed to a slightly higher oil−water partition coefficient than that of chlorogenic acid. The antioxidant activity of the aqueous phase of the Teaw extract was reduced more than that of chlorogenic acid by partitioning of the oil-soluble components into oil, which showed that the less-polar components contributed to the antioxidant activity of the Teaw extract in aqueous media.

KEYWORDS: Antioxidant; oil; emulsion; Teaw.

INTRODUCTION

In general, the inhibitory effect of antioxidants on lipid oxidation is influenced by the physicochemical state of the lipid substrate, and various evaluation systems using different physical conditions are required to provide a good understanding of antioxidant properties in different media (*1*). One of the major factors influencing antioxidant activity in foods is the presence of water, because antioxidants partition between the lipid and aqueous phases, and hydrophilic antioxidants are often less effective in oil-in-water emulsions than lipophilic antioxidants (*2*). To accurately measure the potential for antioxidant activity in foods, models should be developed that have the chemical, physical, and environmental conditions expected in food products. Because these factors vary in food systems, individual models such as bulk oil and emulsion should be studied to allow the antioxidant activity to be assessed.

Antioxidant action becomes more complex in real foods and biological systems where a variety of mechanisms become effective, including free radical chain breaking, oxygen scavenging, singlet oxygen quenching, metal chelation, and inhibition of oxidative enzymes (*3*). Phenolic antioxidants act by donation of a phenolic hydrogen to form an antioxidant radical that is stabilized by electron delocalization and/or intramolecular hydrogen bonding or by further oxidation. The antioxidant activity of many pure phenolic substances has been widely investigated, but the occurrence of mixtures of antioxidant

* Corresponding author. E-mail: m.h.gordon@reading.ac.uk. Telephone: +44-118-3786723. Fax: +44-1189-310080.
† Department of Product Development, Faculty of Agro-Industry, Kasetsart University.
‡ Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, The University of Reading.

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components in plants may be of significance for the food industry, either due to the presence of active components with novel structures or due to synergistic interactions between antioxidants. Therefore, it is important to establish extraction methods that preserve the integrity of the chemical structures and it may also be important to keep phenolic compounds in the same ratios as they are found in the original plant.

Cratoxylum formosum Dyer (Teaw) is an indigenous Thai vegetable that is traditionally consumed as fresh leaves in Thailand. *C. formosum* contains a high concentration of chlorogenic acid (5-*O*-caffeoylquinic acid) (*4*). Chlorogenic acid is widely recognized to be active by free radical scavenging (*5*), and it inhibits peroxidation of linoleic acid (*6*) and acts as a cancer chemopreventive agent (*7*). Teaw is grown on a commercial scale in Thailand, and it is therefore useful to investigate its properties in detail. A previous study demonstrated that the high radical scavenging activity of extracts from *C. formosum* is due to the high content of phenolic compounds, especially chlorogenic acid (*4*). Therefore, this study was designed to compare the antioxidant activity of *C. formosum* extract with chlorogenic acid and standard antioxidants, including α-tocopherol, butylated hydroxytoluene (BHT), ascorbyl palmitate, and citric acid in stripped soybean oil and in an oil-in-water emulsion. The coefficients for partitioning of components between oil and water, and the metal-chelating activity of the plant extract, have also been determined to allow an understanding of the antioxidant activity in model systems.

MATERIALS AND METHODS

Materials. α-Tocopherol, ascorbyl palmitate, acetic acid, chloroform, sodium thiosulfate, potassium iodide, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), chloroform, and butanol were purchased from Fluka Co. (Buchs, Switzerland). Ethanol, 2-propanol, and potassium persulfate were purchased from Sigma (Milwaukee, USA). HPLC-grade methanol and water were purchased from Fisher Scientific (Leicestershire, United Kingdom). Acetonitrile (HPLC grade) and butylated hydroxytoluene (BHT) were purchased from BDH (Poole, United Kingdom). 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1-octanol, citric acid, 1,1,3,3-tetraethoxypropane, Tween 20, isooctane, and 2-propanol were purchased from Sigma-Aldrich Company Ltd. (Gillingham, UK).

Three batches of Teaw leaves (*Cratoxylum formosum* Dyer) were purchased from a farm at Saraburi province, Thailand, during 2004. Immediately on receipt after harvesting, the leaves were washed and sound leaves were selected for extraction.

Soybean oil known to lack added antioxidants was provided by Thai Oil Industry, Bangkok, Thailand. The oil contained 115.7 mg kg^{-1} of α-tocopherol, 918.7 mg kg^{-1} of γ-tocopherol, 228.1 mg kg^{-1} of δ-tocopherol, and the peroxide value determined by AOCS official method cd 8-53 (*8*) was 0.70 ± 0.12 mequiv kg^{-1} oil. The thiobarbituric acid reactive substances value (TBARS), determined according to McDonald et al. (*9*), was 0.0001 mmol kg^{-1} oil.

Preparation of Plant Extracts. Fresh plant leaves (80 g) were blended for 1 min with ethanol at −20 °C, and the containers were then flushed with nitrogen and shaken for 4.5 h in the dark at 25 °C. The supernatant was filtered through cheesecloth and Whatman no. 4 filter paper, and the solvent was evaporated under vacuum. The residue was dried in a freeze-dryer and stored in aluminum foil at −20 °C after flushing with nitrogen.

Preparation of Oil and Oil-in-Water Emulsions. The tocopherols were removed from soybean oil by column chromatography with alumina with some modifications to the method described by Yoshida (*10*). Refined oil (200 g) was passed through a column containing activated aluminum oxide (140 g) dried at 200 °C for 8 h before use. The column was wrapped in aluminum foil to avoid oxidation. The oil was drawn through the column by suction without a solvent. The oil collected was again passed through fresh alumina (140 g) to complete the removal of the tocopherols. The oil was analyzed by HPLC to confirm the removal of the tocopherols. The oil was stored at −70 °C until required for use.

Antioxidants or mixtures of antioxidants were added to stripped soybean oil or 10% oil-in-water emulsion in the following quantities: 100 mg kg^{-1} of crude Teaw extract, α-tocopherol, BHT, ascorbyl palmitate or citric acid, and 60 mg kg^{-1} of chlorogenic acid.

Oil-in-water emulsion (300 g, 10% oil) was prepared by mixing a solution of sodium acetate buffer (267 g, 0.1 M, pH = 5.5) containing Tween 20 (3 g, 1%) and sodium azide to inhibit microbial growth (0.0195 g, 1mM). Stripped soybean oil (30.0 g) containing required antioxidants was added dropwise, as the sample was cooled in an ice bath and homogenized with a sonicator (Branson model 5210, Germany). Sonication was continued for 5 min after the oil had been added. The emulsion was then homogenized with a high-pressure homogenizer (Armfield model FT9, UK) in three cycles at 3000 psi. The droplet size distribution of the emulsion was determined by a static light-scattering technique with a Malvern Instruments particle and droplet sizer (S version 2.19, He−Ne laser source, wavelength 633 nm, beam length 2.40 mm). The mean droplet diameter (μm) in the emulsion was characterized by d_{32}.

$$d_{32} = \sum n_i d_i^3 / \sum n_i d_i^2 \quad (1)$$

where n_i is the number of droplets with diameter d_i. It was found that the mean d_{32} value for the emulsions was 0.42 ± 0.00 μm.

Oxidation and Analysis. Stripped soybean oil and oil-in-water emulsion samples with or without added antioxidant were transferred to screw-capped sample vials with aluminum foil wrapping and held in an oven at 60 °C for 12 days and 9 days, respectively. The lids were only screwed loosely on the vials so that air could pass in and out of the headspace above the samples. Aliquots (10 g) were removed every 3 days for analysis. The oxidative state of samples was monitored by analysis of the peroxide value (PV) and thiobarbituric acid reactive substances (TBARS).

Peroxide value of stripped soybean oil was determined according to AOCS official method Cd 8−53 (*8*) with an automatic titrator (Mettler-Toledo model DL 5X, Switzerland) equipped with stirrer and redox electrode. The solution was titrated against standard sodium thiosulfate (0.01 N). PV was calculated and expressed as mequiv peroxide per kg of oil sample;

$$\text{PV (mequiv/kg)} = \frac{(S - B) \times N \times F}{W} \times 1000 \quad (2)$$

where S is the titer (mL) for the sample, B is the titer (mL) for the blank, N is the normality of the sodium thiosulfate solution, F is the factor from standardization with potassium dichromate, and W the sample weight (g).

Peroxide value of stripped soybean oil-in-water emulsion was determined by adding emulsion (0.3 mL) to isooctane/2-propanol (3:2 v/v, 1.5 mL) followed by vortexing three times for 10 s each. After centrifugation for 2 min at 1000*g*, the clear upper layer (0.2 mL) was collected, and peroxides were quantified by using a method based on that of Diaz et al. (*11*). The sample extract (0.2 mL) was mixed with methanol/1-butanol (2:1 v/v, 2.8 mL) and thiocyanate/Fe^{2+} solution (30 μL) and then vortexed. The thiocyanate/Fe^{2+} solution was prepared by mixing thiocyanate solution (3.94 M) and Fe^{2+} solution (the supernatant of a mixture of equal volumes of 0.144 M $FeSO_4$ and 0.132 M $BaCl_2$ in 0.4 M HCl, 0.072 M). After 20 min of incubation at room temperature, the absorbance was determined at 510 nm. Lipid peroxide concentration was determined by using a cumene hydroperoxide standard curve.

TBARS of oil and emulsion were determined according to McDonald et al. (*9*). Oil (0.1 mL) or emulsion (1 mL) was mixed with water (0.9 mL) and TBA reagent (2.0 mL, 15% w/v trichloroacetic acid and 0.375% w/v thiobarbituric acid in 0.25 M HCl) in test tubes and placed in a boiling water bath for 15 min. The tubes were cooled to room temperature for 10 min and then centrifuged (1000 × *g*) for 15 min. The absorbance was measured at 532 nm. Concentrations of TBARS were determined from a standard curve prepared by using 1,1,3,3-tetraethoxypropane.

Effects of Ferric Ions on Antioxidant Activity. The chelation of ferric ions by the Teaw extract, chlorogenic acid, α-tocopherol, and citric acid was also investigated. Metal ions were removed from stripped soybean oil (100 g) by washing with 5% citric acid (100 mL) in a

separating funnel. This was repeated three times, and the oil was then washed with water (100 mL) twice and saturated sodium chloride solution (100 mL). The oil was then dried with anhydrous sodium sulfate (5 g). Ferric chloride (5 mM) and each antioxidant were added into the stripped, deionized soybean oil. Each sample was stored in a screw-capped sample vial wrapped in aluminum foil in an oven at 60 °C for 48 h. Aliquots (10 g) were removed every 2 h for analysis. The oxidative state of the stripped soybean oil sample was monitored by analysis of the peroxide value (PV) and thiobarbituric acid reactive substances (TBARS). The storage was performed in triplicate for each sample.

Determination of Oil−Water Partition Coefficient. The partition coefficients of the Teaw extract, α-tocopherol, BHT, and chlorogenic acid partitioned between soybean oil and water were determined. Soybean oil (2 g) was weighed into a screw-capped centrifuge tube containing test samples dissolved in HPLC water (2 g) for determination of the partition coefficients of plant extract and chlorogenic acid. The sample was vortexed three times for 20 s with a 20 s interval. The samples were then centrifuged at 6000 rpm for 30 min at 20 °C in a refrigerated centrifuge (Sorvall model RC5B, USA). The lower layer was removed from the centrifuge tube with a syringe. The concentrations of the phenolic components in the aqueous phase were determined quantitatively by reversed-phase HPLC. Samples were filtered through a 0.20 μm Millipore filter (type HA) into a 2-mL autosampler vial for subsequent analysis by HPLC. The solution (10 μL) was injected into the HPLC and analyzed according to the following conditions: column, Synergi Hydro RP column (150 mm × 4.6 mm id., 4 μm, Phenomenex, Macclesfield, UK), fitted with a Allsphere ODS-2 guard column (10 mm × 4.6 mm id., Alltech). Solvent A was 100% acetonitrile. Solvent B was 1% formic acid in water. The program was isocratic at 10% A, 90% B for 10 min, followed by a linear gradient from 10 to 40% A for 39 min and an isocratic period at 10% A, 90% B for 10 min. The flow rate was 0.5 mL/min^{-1}. The HPLC system was equipped with a diode array detector (Dionex PDA 100 photodiode array, USA) controlled by Chromeleon software version 6.60 Build 1428 (Dionex Corporation, Sunnyvale, USA). Chromatograms were monitored at 280 nm. The concentration of each phenolic compound in the plant extract was quantified by using gallic acid as an external standard. The concentration of each phenolic compound in the oil phase (C_{oil}) was calculated as the difference between the total amount of antioxidant in the water before mixing and the amount in the water after mixing with oil (C_{water}). The partition coefficient (log P) was calculated as log(C_{oil}/C_{water}).

BHT and α-tocopherol were dissolved in oil instead of water before mixing of the phases. After separation of the water and oil layers by centrifugation as described above, the concentration of these substances was analyzed in the oil phase by normal phase HPLC. The analysis of α-tocopherol was carried out on an HP series 1100 chromatograph (Hewlett-Packard, Avondale, PA) with detection at 292 nm. A normal-phase silica column (250 mm × 8 mm id., 5 μM, Spherisorb 5 Silica 5U) was used with the mobile phase, hexane/2-propanol (99.5:0.5) at a flow rate of 1 mL/min. Peaks were recorded and integrated by using the HP Chemstation chromatography data system. Partition coefficients (log P) were calculated as above.

Assessment of Free Radical Scavenging Activity. The total free radical scavenging capacity of the aqueous layer of each antioxidant after partition was determined by using the ABTS method. The ABTS radical scavenging activity was determined according to Re et al. (*12*). Briefly, a mixture of 7 mM ABTS and 2.45 mM potassium persulfate was prepared and allowed to stand at 25 °C for 12−16 h in the dark. The $ABTS^{+\bullet}$ solution was diluted to an absorbance of 0.70 (± 0.02) at 734 nm in ethanol before use. $ABTS^{+\bullet}$ solution (2 mL) was added to 20-μL aliquots of Trolox or sample in water with different concentrations. The activity of each antioxidant was determined within the range of the dose−response curve of Trolox, and the radical-scavenging activity was expressed as the Trolox equivalent antioxidant capacity (TEAC), defined as mmol of Trolox per gram of sample.

Statistical Analysis. Each experiment, from sample preparation to analysis, was repeated in triplicate, and the data were then analyzed by SPSS software program (SPSS Inc., Chicago, IL). The general linear model procedure was applied and Duncan's multiple range test was used to compare the mean values at $P < 0.05$. Mean values and pooled standard error of the mean (SEM) were calculated.

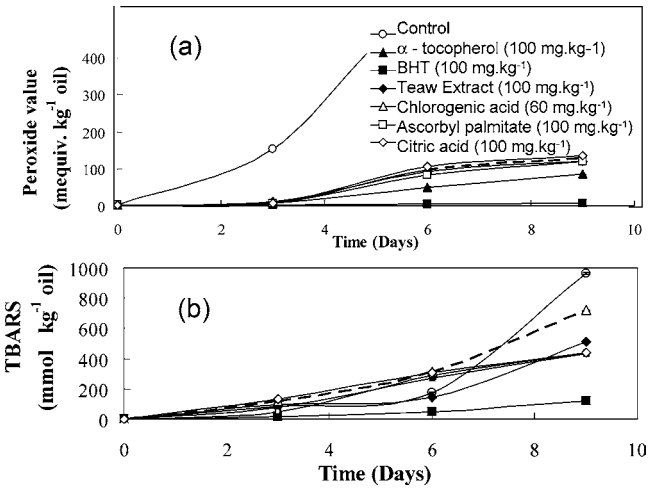

Figure 1. Effect of antioxidants on oxidative stability of stripped soybean oil at 60 °C, assessed by determination of (**a**) PV and (**b**) TBARS. Data points represent mean ± standard deviation ($n = 3$).

RESULTS AND DISCUSSION

Variability of Teaw Leaves. Three batches of Teaw leaves were extracted. The samples were very similar, with the yield = 4.25 ± 0.13% and total phenolics = 66.3 ± 0.6%.

Antioxidant Activity of Phenolic Antioxidants in Stripped Soybean Oil. Soybean oil was used to investigate the antioxidant activity of the Teaw extract and the standard antioxidants because it contains a significant concentration of α-linolenic acid, which makes it quite sensitive to oxidation (*13*). Tocopherols were removed from the soybean oil before use to get a consistent source of oil and to allow primary antioxidant effects of additives to be studied without interference from the tocopherols. Stored samples were analyzed periodically for PV and TBARS to allow both hydroperoxides and hydroperoxide degradation products to be monitored. Volatile hydroperoxide degradation products contribute oxidative off-flavors to foods, and consequently, it is important to monitor both precursors of these off-flavors as well as the degradation products themselves. Secondary oxidation products include aldehydes, ketones, hydrocarbons, and alcohols, among others. The detection of malonaldehyde (MDA), a volatile dialdehyde, has been widely used as a measure of the oxidation of polyunsaturated fatty acids in foods and animal tissues. It is a secondary product of autoxidation of fatty acids having three or more double bonds, and the TBAR values that are a measure of malonaldehyde have been shown to correlate well with flavor threshold values for some vegetable oils (*14*).

Changes during storage in the peroxide values of stripped soybean oil containing Teaw extract compared with oil containing individual antioxidants were investigated (**Figure 1a**). Because the Teaw extract has been shown to contain about 60% chlorogenic acid as the main antioxidant component (*4*), the concentration of chlorogenic acid used for comparison was 60 mg kg^{-1}. On the basis of the time to a PV of 50 mequiv kg^{-1} (**Table 1**), antioxidant efficiency for 100 mg kg^{-1} addition decreased in the following order: citric acid > BHT > ascorbyl palmitate > chlorogenic acid (60 mg kg^{-1}) > Teaw extract > α-tocopherol > control.

The TBARS determinations (**Figure 1b**) confirmed that α-tocopherol was the weakest antioxidant in oil, but BHT was less effective at inhibiting formation of TBARS than chlorogenic acid, whereas it was more effective at reducing the rate of hydroperoxide formation. The ratio of PV/TBARS for the different samples is shown in **Figure 2**. The ratio decreased

Table 1. Time for Stripped Soybean Oil and Oil-in-Water Emulsion Samples Containing Antioxidants (100 mg kg^{-1}) to Reach a Peroxide Value of 50 mequiv kg^{-1} at 60 °C[a]

	bulk oil			10% oil-in-water emulsion		
compound	time (days)	R^2	PF[c]	time (days)	R^2	PF[c]
control	3.27 ± 0.01 a	0.99	1.00 ± 0.00 a	1.56 ± 0.11 a	1.00	1.00 ± 0.00 a
α-tocopherol	4.81 ± 0.01 b	0.97	1.47 ± 0.00 b	6.52 ± 0.07 e	0.97	4.19 ± 0.07 c
BHT	8.80 ± 0.02 f	0.97	2.69 ± 0.01 f	14.73 ± 0.01 f	0.98	9.45 ± 0.07 d
Teaw extract	6.06 ± 0.01 c	0.99	1.86 ± 0.00 c	4.55 ± 0.02 c	0.96	2.92 ± 0.02 b
chlorogenic acid[b]	6.18 ± 0.02 d	0.99	1.89 ± 0.01 d	4.44 ± 0.10 c	0.98	2.84 ± 0.04 b
ascorbyl palmitate	7.84 ± 0.01 e	0.99	2.40 ± 0.01 e	5.00 ± 0.06 d	0.97	3.21 ± 0.09 b
citric acid	9.29 ± 0.00 g	0.98	2.84 ± 0.01 g	4.25 ± 0.03 b	0.97	2.73 ± 0.01 b

[a] Data followed by different letters within each column are significantly different according to Duncan's multiple range test at $P < 0.05$. Data are shown as means ± standard deviation from three replicate determinations. [b] Concentration of chlorogenic acid was 60 mg kg^{-1}. [c] PF = [days to reach PV 50 mequiv kg^{-1} for each antioxidant/days to reach PV 50 mequiv kg^{-1} for control (no antioxidant)].

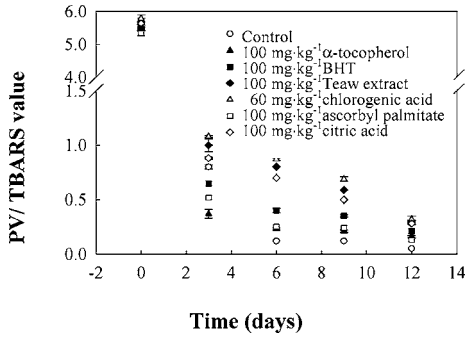

Figure 2. The ratio of peroxide and TBARS values (PV/TBARS value) of stripped soybean oil containing antioxidants during storage at 60 °C. Data points represent mean ± standard deviation ($n = 3$).

during storage, but it was higher for the samples containing chlorogenic acid, Teaw extract, and citric acid than for the samples that contained BHT, α-tocopherol, or ascorbyl palmitate throughout the storage period. Satue et al. (*15*) reported that antioxidants showed different activities toward hydroperoxide decomposition. For the antioxidants used in this study, the differences in the relative values for hydroperoxides and TBARS values are expected to relate to the metal-chelating ability of the antioxidant. Metals are known to catalyze hydroperoxide decomposition, which leads to the formation of the aldehydes and related compounds that are determined in the TBARS assay. Chlorogenic acid is known as a metal chelator (*16*), and the Teaw extract contained 60% chlorogenic acid. Consequently, it is consistent with the known metal-chelating activity of chlorogenic acid that the PV/TBARS ratio for oil samples containing chlorogenic acid should be higher than that for α-tocopherol and BHT, which do not have the *o*-diphenol structure necessary for molecules to chelate metal ions. A further experiment was performed in order to investigate whether this explanation was valid.

Citric acid-washed, stripped soybean oil with and without added ferric chloride was used to study the effect of antioxidants (α-tocopherol, chlorogenic acid, and citric acid) on hydroperoxide and TBARS formation in oil with a low and a high metal ion content. The ratio of PV/TBARS for the metal-free samples is shown in **Figure 3a**. In the oil without added iron, the ratio remained about the same for the citric acid and control samples during storage or tended to increase with longer storage times for the chlorogenic acid and α-tocopherol samples. This indicates that the radical-scavenging antioxidants were inhibiting hydroperoxide decomposition and causing some increase in the PV/TBARS ratio during storage if metal ions were absent. The PV values of the samples at 0, 12, 24, 36, and 48 h are given in **Table 2**. There was no significant difference ($P \geq 0.05$) in

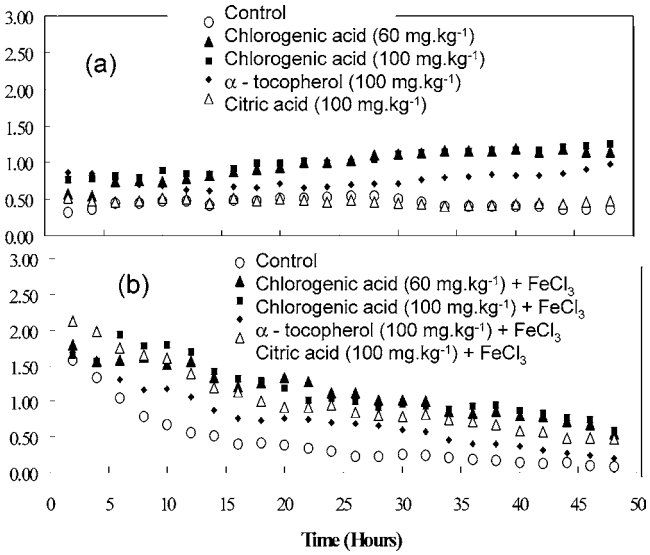

Figure 3. The ratio of peroxide and TBARS values (PV/TBARS value) of studied antioxidants on oxidative stability of (**a**) citric acid-washed stripped soybean oil and (**b**) stripped soybean oil containing added ferric ions at 60 °C. Data points represent mean ± standard deviation ($n = 3$).

the PV/TBARS ratio for samples containing chlorogenic acid at 60 and 100 mg kg^{-1}. The PV/TBARS ratio was higher for chlorogenic acid than that for α-tocopherol.

The ratio of PV/TBARS during storage was quite different in the oil containing added ferric ions (**Figure 3b**) from that in the metal-free oil. The ratio was high initially, but it fell progressively during storage. The ratio was highest in the order chlorogenic acid > citric acid > tocopherol > control. Antioxidants can promote oxidation in the presence of ferric ions under certain conditions by reducing the ferric to the ferrous ion, which is more active in catalyzing decomposition of hydroperoxides, but this is an ionic reaction that occurs mainly in aqueous systems (*2*). In oils, antioxidants may chelate the metal and thereby retard oxidation. In this experiment, the PV and TBARS values were higher for the control and antioxidant samples in the oil containing added ferric ions than that for the oil lacking ferric ions (**Table 2**). This shows that the metal was catalyzing both hydroperoxide formation and hydroperoxide decomposition.

Chlorogenic acid can function both as a radical scavenger and as a metal chelator due to the presence of an *ortho*-dihydroxy grouping in its chemical structure (*16*), whereas citric acid can only act as a metal chelator. However, citric acid is a more effective metal chelator than chlorogenic acid, and hence citric acid is more effective at inhibiting increase in the TBARS value due to metal-catalyzed hydroperoxide decomposition, as

Table 2. Peroxide and TBARS Values for Samples of Citric Acid-Washed Oil and Oil Containing Added Ferric Ions Plus Antioxidants (100 mg kg^{-1}) at 60 °C[a]

		oil with no added ferric ions		oil with added ferric ions	
compounds	time (hours)	PV (mequiv kg^{-1})	TBARS (mmol kg^{-1})	PV (mequiv kg^{-1})	TBARS (mmol kg^{-1})
control	0	1.48 ± 0.05 a	0.24 ± 0.03 a	1.48 ± 0.05 a	0.24 ± 0.03 a
	12	6.80 ± 0.11 c	14.25 ± 0.14 e	8.93 ± 0.05 f	25.57 ± 0.29 h
	24	11.39 ± 0.07 h	21.76 ± 0.54 h	15.42 ± 0.04 ij	52.01 ± 0.29 l
	36	15.82 ± 0.07 j	40.01 ± 0.47 j	33.84 ± 0.66 o	183.44 ± 0.92 o
	48	22.72 ± 0.34 k	46.40 ± 0.15 l	59.41 ± 2.12 q	748.87 ± 1.76 q
α-tocopherol	0	1.48 ± 0.05 a	0.24 ± 0.03 a	1.48 ± 0.05 a	0.24 ± 0.03 a
	12	5.09 ± 0.08 b	8.12 ± 0.07 cd	6.79 ± 0.04 c	6.42 ± 0.01 b
	24	8.01 ± 0.47 de	11.93 ± 0.58 ef	14.35 ± 0.04 i	20.31 ± 1.35 c
	36	10.37 ± 0.25 h	12.74 ± 0.00 fg	25.49 ± 0.50 m	63.38 ± 0.10 n
	48	14.20 ± 0.25 i	14.51 ± 0.08 gh	41.43 ± 0.63 p	211.09 ± 1.25 p
chlorogenic acid[b]	0	1.48 ± 0.05 a	0.24 ± 0.03 a	1.48 ± 0.05 a	0.22 ± 0.00 a
	12	5.41 ± 0.27 b	6.94 ± 0.01 b	5.08 ± 0.03 b	3.25 ± 0.03 b
	24	7.70 ± 0.19 d	7.62 ± 0.03 c	10.09 ± 0.03 gh	9.03 ± 0.01 c
	36	9.38 ± 0.16 fg	8.11 ± 0.00 c	15.67 ± 0.30 j	18.91 ± 0.38 h
	48	10.78 ± 0.14 h	9.41 ± 0.18 c	29.66 ± 1.08 n	54.87 ± 1.41 m
chlorogenic acid	0	1.48 ± 0.05 a	0.24 ± 0.03 a	1.48 ± 0.05 a	0.23 ± 0.01 a
	12	4.66 ± 0.07 b	5.97 ± 0.03 b	4.80 ± 0.22 b	2.83 ± 0.03 b
	24	7.30 ± 0.52 cd	7.33 ± 0.04 c	9.91 ± 0.33 gh	9.43 ± 0.02 c
	36	9.13 ± 0.29 f	7.86 ± 0.01 c	15.01 ± 0.29 ij	16.00 ± 0.03 h
	48	10.82 ± 0.11 h	8.60 ± 0.01 c	24.04 ± 0.88 l	43.50 ± 1.23 k
citric acid	0	1.48 ± 0.05 a	0.24 ± 0.03 a	1.48 ± 0.05 a	0.24 ± 0.03 a
	12	6.94 ± 0.14 c	13.65 ± 0.41 de	4.51 ± 0.24 b	3.24 ± 0.03 b
	24	10.69 ± 0.20 h	22.97 ± 0.54 h	8.66 ± 0.28 ef	9.13 ± 0.02 c
	36	15.51 ± 0.25 j	37.58 ± 0.51 i	14.68 ± 0.29 i	19.50 ± 0.14 h
	48	21.50 ± 0.21 k	45.18 ± 0.15 l	22.14 ± 0.14 k	41.91 ± 1.17 j

[a] Data followed by different letters within each column are significantly different according to Duncan's multiple range test at $P < 0.05$. Data were represented as means from three replication measurement. [a] Concentration of chlorogenic acid was 60 mg kg^{-1}.

seen in the low TBARS value for citric acid in oil with added ferric ions (**Table 2**). This experiment confirms that the metal-chelating activity of chlorogenic acid plays an important role in the ability of the antioxidant to inhibit decomposition of hydroperoxides in oils. Hence, the antioxidant is effective in retarding development of off-flavors, not only because of its radical-scavenging activity, which retards formation of hydroperoxides, but also because of its metal-chelating activity, which retards decomposition of hydroperoxides.

In a previous study (*4*), we found that the Teaw extract contained 60% chlorogenic acid together with minor components, which included dicaffeoylquinic acid and ferulic acid derivatives, at a level of approximately 12% when quantified by HPLC as chlorogenic acid equivalents. In this study, the antioxidant effect of Teaw extract in stripped soybean oil at 100 mg kg^{-1} was similar to that of chlorogenic acid at 60 mg kg^{-1}. This study confirmed that chlorogenic acid was the main antioxidant in the Teaw extract, and the other components do not contribute significantly to the antioxidant activity of the extract in oil. The minor components in the Teaw extract elute later than chlorogenic acid in reversed-phase HPLC and hence are less polar. The poor antioxidant activity of α-tocopherol and the minor components of the Teaw extract compared to chlorogenic acid is in agreement with the polar paradox, which concludes that less-polar antioxidants are less effective than polar antioxidants in an oil medium (*17*).

Antioxidant Activity of Phenolic Antioxidants in Stripped Soybean Oil-in-Water Emulsions. The effect of the Teaw extract and the standard antioxidants on the oxidative stability of an emulsion comprising 10% stripped soybean oil-in-water during storage at 60 °C in the dark was studied by monitoring the PV and TBARS values. The emulsions were physically stable during 9 days of incubation. The size of the oil droplets in the emulsion was 0.42 ± 0.00 μm, and no creaming, flocculation, coalescence, or oil separation was observed. This is within the recommended range of <1.00 μm (*1*), which is significant because the droplet size of the emulsion can affect the physical stability of the emulsion and the lipid oxidation rate. The formation of hydroperoxides increased significantly more in the control emulsion than in the emulsion with individual antioxidant added and stored at 60 °C (**Figure 4a**). Oxidation was more rapid in stripped soybean oil-in-water emulsion than in stripped soybean oil, as is commonly found when comparing oxidation in oil and emulsions (*18*).

The order of antioxidant activity in the oil-in-water emulsion was different from that in bulk oil. On the basis of the time to a PV of 50 mequiv kg^{-1}, the order of antioxidant activity (**Table 1**) was BHT (100 mg kg^{-1}) > α-tocopherol (100 mg kg^{-1}) > ascorbyl palmitate (100 mg kg^{-1}) > Teaw extract (100 mg kg^{-1}), chlorogenic acid (60 mg kg^{-1}) > citric acid. The TBARS values confirmed the order of activity (**Figure 4b**). The TBARS values increased more rapidly for the control samples in the emulsion system (**Figure 4b**) than in the oil system (**Figure 1**), with a TBARS value of 200 mmol kg^{-1} being reached in about 4 days in the emulsion compared to >5 days in the oil. It appears likely that when hydroperoxides are formed in an emulsion, they are concentrated at the oil−water interface where they are subject to further degradation by radicals generated in the aqueous phase or by metals.

The changes in the ratio of the PV/TBARS values with time are shown in **Figure 5**. The PV/TBARS ratio of the control sample was highest due to the high level of hydroperoxide formation in the emulsion. Citric acid showed the lowest PV/TBARS ratio. BHT was the most effective antioxidant overall considering the results from both the oil and emulsion studies. The tertiary butyl group of BHT is effective in slowing reactions at the active −OH group by steric hindrance and thereby increasing the stability of the antioxidant radical and extending the active life of the antioxidant (*19*). Chlorogenic acid was

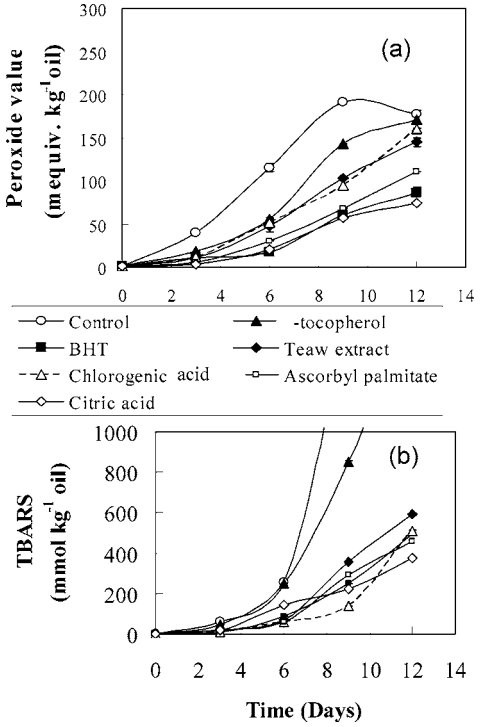

Figure 4. Effect of studied antioxidants on oxidative stability of stripped soybean oil-in-water emulsion at 60 °C, assessed by determination of (**a**) PV and (**b**) TBARS. Data points represent mean ± standard deviation ($n = 3$)

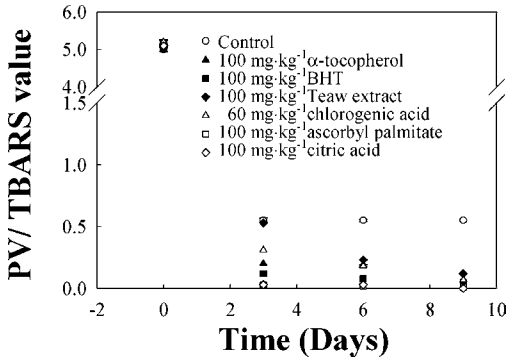

Figure 5. The ratio of peroxide and TBARS values (PV/TBARS value) of stripped soybean oil-in-water emulsions containing antioxidants during storage at 60 °C. Data points represent means ($n = 3$) ± standard deviation.

relatively ineffective as an antioxidant in the emulsion because of its polarity.

The PV/TBARS ratios for the samples containing antioxidants fell rapidly to lower values (<0.3) in the emulsion (**Figure 5**) than in the oil (**Figure 2**). Trace levels of metals are naturally present in oil and water. Metals are more active at catalyzing hydroperoxide decomposition in emulsions, and the catalytic effect of the naturally occurring metal ions can partly explain the rapid fall in PV/TBAR ratio in the emulsion. It appears likely that when hydroperoxides are formed in an emulsion, they are located at the oil−water interface where they are subject to further degradation by radicals generated in the aqueous phase or by metals. This leads to a more rapid increase in TBARS than in PV. It was not thought useful to investigate the effect of adding higher levels of metal ions to the emulsion because it is known that relatively high levels of metals rapidly degrade phenolic components in emulsions (*2*).

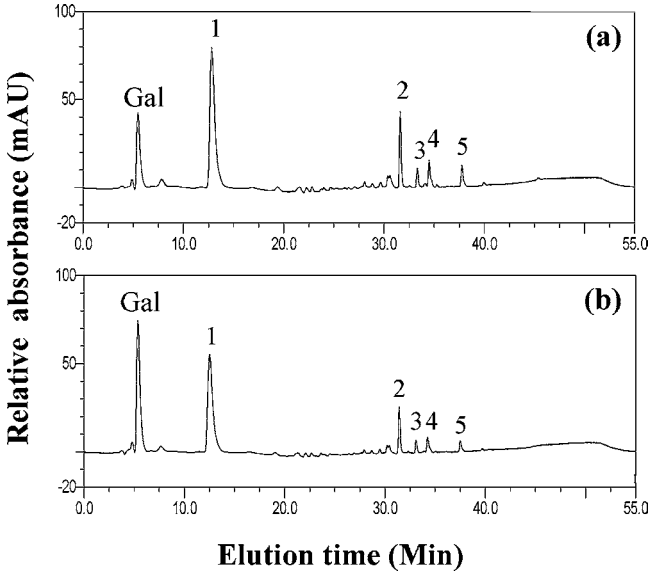

Figure 6. HPLC chromatogram of phenolic compounds in the Teaw extract (**a**) before and (**b**) after partitioning into oil (Gal = gallic acid added to the sample as an internal standard; 1 = chlorogenic acid, RMM 354, and λ_{max} 325 nm; 2 = dicaffeoylquinic acid, RMM 354, and λ_{max} 325 nm; 3 = ferulic acid derivative with λ_{max} 312–315 nm; 4 = ferulic acid hexose derivative with RMM 452 and λ_{max} 312–315 nm; 5 = component with λ_{max} 310 nm).

Table 3. Oil–Water Partition Coefficient (log *P*) and $ABTS^{+\bullet}$ Radical Scavenging Activity of Teaw and Chlorogenic Acid[a]

		$ABTS^{+\bullet}$ radical scavenging activity	
compound	log P^{ns}	stock solution	aqueous phase after breaking emulsion
Teaw	−0.67 ± 0.00	2.71 ± 0.06 a	2.33 ± 0.16 a
chlorogenic acid	−0.77 ± 0.00	3.06 ± 0.04 b	2.92 ± 0.04 b

[a] Data followed by different letters within each column are significantly different according to Duncan's multiple range test at $P < 0.05$. Nonsignificance is denoted by ns within each column. Data obtained from at least ten replicates for the oil–water partition coefficient and three replicates for the $ABTS^{+\bullet}$ assay.

The polarity of the antioxidants was assessed by determination of the oil−water partition coefficient by HPLC. For the Teaw extract, the oil−water partition coefficient was calculated by summing the areas of the five phenolic peaks in the HPLC chromatogram of an aqueous solution of the Teaw extract compared with the areas of the five peaks in the aqueous phase after preparation and breaking of an emulsion. The relative areas of the HPLC peaks of the Teaw extract in the aqueous phase before and after partitioning with oil changed, as shown in **Figure 6**. The oil−water partition coefficient of the Teaw extract and chlorogenic acid were very similar, as shown in **Table 3**. This was expected because a previous study showed that chlorogenic acid was the main component in the Teaw extract at a concentration of 60% of the extract and three minor phenolic components were present at a level of 12%. The Teaw extract had a slightly higher partition coefficient than chlorogenic acid because of the presence of the minor components, including dicaffeoylquinic acid and ferulic acid derivatives, which were less polar than chlorogenic acid.(*4*). α-Tocopherol and BHT were completely insoluble in water because they are much less polar. Partition coefficients (log *P*) of ascorbyl palmitate (*20*) and citric acid (*21*) were reported as 0.719 and −1.64, respectively, compared with values of −0.68 for the Teaw extract and −0.77 for chlorogenic acid. Therefore, citric acid

is more polar than the other studied antioxidants. By comparing the order of activity as antioxidants, the finding that chlorogenic acid and the Teaw extract were more effective than α-tocopherol in inhibiting lipid oxidation in bulk oil but were less effective in an oil-in-water emulsion is consistent with the previous literature. This phenomenon was described as the "polar paradox" (*17*). This has been explained as being due to the nonpolar antioxidant being concentrated at the oil−water interface, where they are effective at preventing reaction of triacylglycerols with radicals generated in the aqueous phase (*22*). In the emulsion, polar antioxidants would tend to partition into the aqueous phase, where they would not be able to protect the lipid effectively. The Teaw extract at 100 mg kg^{-1} is more effective than chlorogenic acid (60 mg kg^{-1}) in an emulsion, but it is less effective in oil (**Table 1**), which is also consistent with the Teaw extract containing some less-polar antioxidants that contribute particularly in the emulsion system.

The change in antioxidant activity of the aqueous phase analyzed directly without any oil addition and after breaking the emulsion was determined by the $ABTS^{+\bullet}$ radical scavenging assay (**Table 2**). The fall in the radical scavenging capacity of the aqueous phase after emulsion preparation was greater for the Teaw extract than for the chlorogenic acid solution, which confirms that the less-polar components of the Teaw extract contribute to the radical scavenging activity in water, but partition more effectively into the oil phase than chlorogenic acid in the emulsion.

In conclusion, chlorogenic acid and the Teaw extract were more effective than α-tocopherol in inhibiting lipid oxidation in bulk oil but were less effective in an oil-in-water emulsion. The importance of the metal-chelating activity in retarding hydroperoxide decomposition was confirmed by studying the decomposition of oil samples containing added ferric ions. The PV/TBARS ratio was higher for citric acid than for α-tocopherol in the presence of added ferric chloride, but the order was reversed in samples washed with citric acid to remove metal ions. Samples containing added chlorogenic acid gave the highest PV/TBARS ratios both in the presence and absence of ferric ions. The PV/TBARS ratios for the samples containing antioxidants fell rapidly to lower values in the emulsion than in the oil. This was due to a more rapid increase in TBARS than in PV due to increased hydroperoxide decomposition in the emulsion. The Teaw extract contained 12% oil-soluble components, which contributed to a slightly higher oil−water partition coefficient than that of chlorogenic acid. The antioxidant activity of the aqueous phase of the Teaw extract was reduced more than that of chlorogenic acid by partitioning of the oil-soluble components into oil, which showed that the less-polar components could contribute to the antioxidant activity of the Teaw extract.

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