Antioxidant Properties of Water Extracts from *Cassia tora* L. in Relation to the Degree of Roasting

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The antioxidant properties of water extracts from *Cassia tora* L. (WECT) prepared under different degrees of roasting were investigated. The water extracts of unroasted *C. tora* L. (WEUCT) showed 94% inhibition of peroxidation of linoleic acid at a dose of 0.2 mg/mL, which was higher than that of α-tocopherol (82%). Water extracts prepared from *C. tora* L. roasted at 175 °C for 5 min and at 200 °C for 5 min exhibited 83% and 82%, respectively, inhibition of linoleic acid peroxidation. This result indicated that the antioxidant activities of WECT decreased with longer roasting time or higher roasting temperature. The IC_{50} of WEUCT in liposome oxidation induced by the Fenton reaction was 0.41 mg/mL, which was higher than that of α-tocopherol (IC_{50} = 0.55 mg/mL). WEUCT also exhibited good antioxidant activity in enzymatic and nonenzymatic microsome oxidative systems. The water extracts of roasted *C. tora* L. increased in the degree of browning and produced chemiluminescence when compared with the unroasted sample. However, the total polyphenolic compounds of WECT decreased after the roasting process finished. In conclusion, the decrease in the antioxidant activity of water extracts from roasted *C. tora* L. might have been due to the degradation of Maillard reaction products and the decrease of polyphenolic compounds.

Keywords: *Cassia tora; water extract; antioxidant activity; roasting; browning; polyphenol*

INTRODUCTION

There is a considerable amount of evidence revealing an association between individuals who have a diet rich in fresh fruit and vegetables and a decreased risk of cardiovascular diseases and certain forms of cancer (Salah et al., 1995). It is generally assumed that the dietary elements responsible for these protective effects are antioxidant nutrients. However, more recent work has highlighted the additional role of the polyphenolic components of the higher plants (Hertog et al., 1993), which may act as antioxidants or agents of other mechanisms that contribute to their anticarcinogenic or cardioprotective actions. Several studies have shown that increased dietary intake of natural phenolic antioxidants correlates with reduced coronary heart disease (Stampfer et al., 1993). Recent epidemiological studies have suggested that diets rich in phenolic compounds are associated with longer life expectancy (Hertog and Hollman, 1996). These compounds have also been found to exhibit many health-related properties because of their antioxidant activities. These properties include anticancer, antiviral, and antiinflammatory activities, effects on capillary fragility, and an ability to inhibit human platelet aggregation (Benavente-Garcia et al., 1997).

"Jue-ming-zi", the seed of *Cassia tora* L. (Leguminosae), has been used as a laxative and a tonic Chinese herb for several centuries. Su (1992) and Kim (1994) reported that methanolic extracts from *C. tora* L. exhibited a strong antioxidant activity on lipid peroxidation. Yen et al. (1998) also indicated that the antioxidant activity of methanolic extracts from *C. tora* L. was stronger than that of *C. occidentalis* L., and they also identified an antioxidative compound as 1,3,8-trihydroxy-6-methyl-9, 10-anthracenedione (emodin) from *C. tora*. The commercial products of *C. tora* include both unroasted and roasted samples, and the laxative effect was found to be higher in unroasted *C. tora* than in the roasted product. Roasted *C. tora* has a special flavor and color, and it is popularly used to make a health drink. Zhang et al. (1996) reported that some components, e.g., chrysophanol, in *C. tora* were decreased after the roasting process. Nicoli et al. (1997) found that medium-dark roasted coffee brews had the highest antioxidant properties due to the development of Maillard reaction products. In view of this, the antioxidant activity of *C. tora* might also be influenced by the roasting treatment. However, there are no standard conditions for roasting *C. tora*, and the data concerning the effect of different degrees of roasting on the antioxidant properties of *C. tora* are not available. Thus, the objectives of this study were to investigate the effects of roasting conditions on the antioxidant properties of water extracts from *C. tora* and to find the relationship between antioxidant activity and the degree of roasting of *C. tora*.

MATERIALS AND METHODS

Material and Chemicals. The seeds of *C. tora* L. were purchased at a local market in Taichung, Taiwan. Linoleic acid, α-tocopherol, butylated hydroxyanisole (BHA), ascorbic acid, trolox, adenosine 5′-diphosphate (ADP), and lucigenin were purchased from Sigma Chemical Co. (St. Louis, MO). Ammonium thiocyanate, iron chloride tetrahydrate, sodium dihydrogen phosphate, and disodium hydrogen phosphate were purchased from Hayashi Chemical Co. (Tokyo, Japan). Egg lecithin, thiobarbituric acid (TBA), dihydronicotinamid-adenindinucleotide (NADH), and tricholoracetic acid (TCA) were purchased from E. Merck Co. (Darmstadt, Germany).

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Sample Preparation. To obtain *C. tora* L. seeds under different degrees of roasting, washed and sun-dried samples (600 g) were roasted at 150, 175, 200, and 250 °C (internal temperature) for 5 min using a roasting machine (26 cm × 22 cm i.d., rotating rate at 20 rotation/min, Nankung Machine Co., Taiwan). The degree of roasting was classified as minimum roasting (150 °C), medium roasting (175 °C), commercial roasting (200 °C), and over roasting (250 °C). Since the product would become dark when roasted at 200 °C with an increase of roasting time, the effect of roasting time on the antioxidant activity of *C. tora* was studied at 175 °C for 1, 3, 5, 10, and 80 min. Water extracts from *C. tora* were prepared using a modified method of Yen and Chen (1994). Each unroasted and roasted sample (20 g) was extracted with boiling water (200 mL) for 10 min. The extracts were filtered through Whatman no. 1 filter paper, and the filtrates were freeze-dried. The water extract of *C. tora* L. was named WECT.

Determination of Antioxidant Activity. The antioxidant activity of WECT was determined using the thiocyanate method (Yen and Hsieh, 1998). Each sample (1 mg) in 0.5 mL of deionized water was mixed with linoleic acid emulsion (2.5 mL, 0.02 M, pH 7.0) and potassium phosphate buffer (2 mL, 0.2 M, pH 7.0). The reaction mixture was incubated at 37 °C. Aliquots of 0.1 mL were taken at different intervals during incubation. The degree of oxidation was measured according to the thiocyanate method by sequentially adding ethanol (4.7 mL, 75%), ammonium thiocyanate (0.1 mL, 30%), sample solution (0.1 mL), and ferrous chloride (0.1 mL, 0.02 M in 3.5% HCl). After the mixture was left for 3 min, the peroxide value was determined by reading the absorbance at 500 nm using a spectrophotometer (Hitachi U-2000). A control was performed with linoleic acid but without the extracts. Trolox, BHA, α-tocopherol, and ascorbic acid were used as positive controls.

Liposomes Preparation and Oxidation. Liposomes were prepared according to the method of Tsuda et al. (1994). Egg lecithin (5 g) was dispersed in a sodium phosphate buffer (500 mL, 20 mM, pH 7.4) and sonicated in a Branson sonicator for 30 min. Consequently, small vesicles were obtained. All operations were carried out under N_2, and sonication was done in an ice-cold water bath. Varying concentrations (0.05−1.0 mg/mL) of the extracts were tested for lipid peroxidation activities as follows. The extract (0.5 mL) was mixed with liposomes (2.0 mL), 25 mM $FeCl_3$ (0.1 mL), 25 mM H_2O_2 (0.1 mL), 25 mM ascorbic acid (0.1 mL), and 0.2 M phosphate buffer (1.2 mL, pH 7.4). The reaction mixture was incubated at 37 °C for 4 h. At the end of incubation, 1 mL of BHT (20 mg/mL in methanol) was added to the mixture to stop the oxidation reaction. The extent of oxidation of liposomes was subsequently determined by measuring the thiobarbituric acid-reactive substances (TBARS). The reaction mixture was added with 1 mL each of 1% TBA and 10% HCl, and it was then heated in a water bath at 100 °C for 30 min. After the mixture was cooled in an ice bath for 15 min, 5 mL of chloroform was added, and the mixture was centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was measured spectrophotometrically at 532 nm. Iinhibition percent of TBARS formation of was calculated as $100(A_0 - A_t)/(A_0 - A_1)$, where A_0, A_1, and A_t are the absorbance values for control, for blank, and for test sample added, respectively. Trolox was assayed for comparison of the results.

Liver Microsome Preparation and Assaying the Oxidation Induced by Enzymatic System. Microsomes were prepared according to the method of Hanna et al. (1994). Microsomes were isolated from male Sprague−Dawley rats weighing about 220 g as follows. The liver was sliced and homogenized in 4 volumes of homogenization buffer (1.15% KCl/10 mM potassium phosphate buffer, pH 7.4). The homogenate was centrifuged at 600*g* for 10 min at room temperature, the resulting supernatant was recentrifuged at 4 °C, 9000*g* for 10 min, and the final supernatant was ultracentrifuged at 4 °C, 100000*g* for 45 min. The resulting pellet was resuspended in the homogenization buffer. Liver microsomal oxidation was performed according to a modified method of Tsuda et al. (1994). The extract (0.2 mL, 0.05−0.5 mg/mL) was mixed with liver microsomes (0.2 mL, 0.5 mg protein/mL). The mixtures were incubated in the presence or absence of a reduced NADP (NADPH)-generating system (0.2 mL, 1 mM NADPH; 0.2 mL, 1 mM ADP and 0.2 mL, 0.25 mM $FeCl_3$) in a phosphate buffer (0.2 M, pH 7.4). The total final volume was 1 mL. The reaction mixtures were incubated at 37 °C for 1 h. The extent of oxidation of microsomes was measured by means of the TBARS measurement as described above.

Determination of Browning Intensity. The water extracts of *C. tora* prepared under different degrees of roasting were filtered and adjusted to a final volume 200 mL with distilled water. After dilution with distilled water (1:1, v/v), absorbance of the solution was read at 420 nm, using a Hitachi U3000 spectrophotometer (Hitachi, Tokyo, Japan). A high absorbance value indicated high browning intensity.

Measurement of Chemiluminescene (CL). The chemiluminescene was measured according to a modified method of Chen et al. (1997). WECT (0.5 mL) was mixed with 0.5 mL of distilled water and 0.5 mL of 0.1 mM lucigenin in a sample cell (Amethyst, 5.0 cm i.d. open dish). The cell was then placed in an absolutely dark chamber of a chemiluminescence analyzer (BJL Ultra-Weak Chemiluminescence, Jye Horn Business Co., Taipei, Taiwan). The sample was measured continuously for a total of 600 s. The total CL was calculated by integrating the area under the curve. The data were expressed as CL counts/10 s.

Determination of Total Phenolics Content. The total content of phenolics was determined according to the method of Taga et al. (1984) and calculated using gallic acid as a standard. WECT powder (10 mg) was dissolved in 5 mL of acidified methanol/water (60/40, 0.3% HCl). The resulting solution (100 μL) was added to 2 mL of 2% Na_2CO_3. After 2 min, 50% Folib−Cioalteu reagent (100 μL) was added to the mixture, which was then left for 30 min. Absorbance was measured at 750 nm using a spectrophotometer. Results were expressed as miligrams per gram of Gallic acid/g WECT.

Analysis of Amino Acid Composition. The sample was first hydrolyzed with 6 N HCl in a vacuum at 110 °C for 24 h. The amino acids in the hydrolysates were determined by means of HPLC and precolumn derivatization with *o*-phthaldialdehyde (OPA). A Hitachi L-6200 liquid chromatograph equipped with a Lichrospher 100 RP-18 column (125 × 4 mm, 5 μm, E. Merck) and a fluorescence detector (Hitachi F1300, excitation at 340 nm, emission at 450 nm) was used. The mobile phase used for the separation of OPA-amino acid consisted of two eluents: 50 nm sodium acetate, pH 5.7, containing 0.5% tetrahydrofuran (solvent A); and methanol (solvent B). The gradient profiles were as follows: 0−5 min, 17% B; 5−8 min, 17−24% B; 8−14 min, 24% B; 14−23 min, 24−50% B; 23−28 min, 50% B; 28−34 min, 50−67% B; and 34−34.5 min, 67% B. A 10 μL portion of the derivative was injected into the HPLC at a constant flow rate of 1.2 mL/min, with the column temperature maintained at 37 °C.

Statistical Analysis. All results were run in three replicates and averaged. Data were analyzed using the Statistical Analysis System software package (SAS, 1985). Analyses of variance were performed using ANOVA procedures. Significant differences ($P < 0.05$) between means were determined by means of Duncan's multiple range test.

RESULTS AND DISCUSSION

The Yields of WECT with Different Degrees of Roasting. Table 1 shows the yield and extraction ratio of water extracts from *C. tora* with different degrees of roasting. The yield of extracts increased with an increase in roasting temperature but decreased at roasting temperature over 200 °C. The extraction ratio of unroasted *C. tora* was 2.58%, while it increased to 7.71% when roasted at 170 °C for 10 min. The higher roasting temperature increased the browning of *C. tora* that could form nonsoluble products and cause the decrease in extraction yield.

Antioxidant Activity of WECT in the Linoleic Acid Peroxidation System. From the results shown

Table 1. Yield and Ratio of Water Extracts from *C. tora* L. with Different Degrees of Roasting

sample[a]	yield (g)	ratio (%)
unroasted	0.52	2.58
150 °C, 5 min	0.93	4.65
175 °C, 1 min	1.14	5.71
3 min	1.26	6.33
5 min	1.36	6.86
10 min	1.54	7.71
80 min	1.48	7.43
200 °C, 5 min	0.57	2.89
250 °C, 5 min	0.51	2.55
commercial product	1.49	7.48

[a] Sample (20 g) was extracted with boiling water (200 mL) for 10 min. The extracts were filtered and freeze-dried. Data are the average of two experiments.

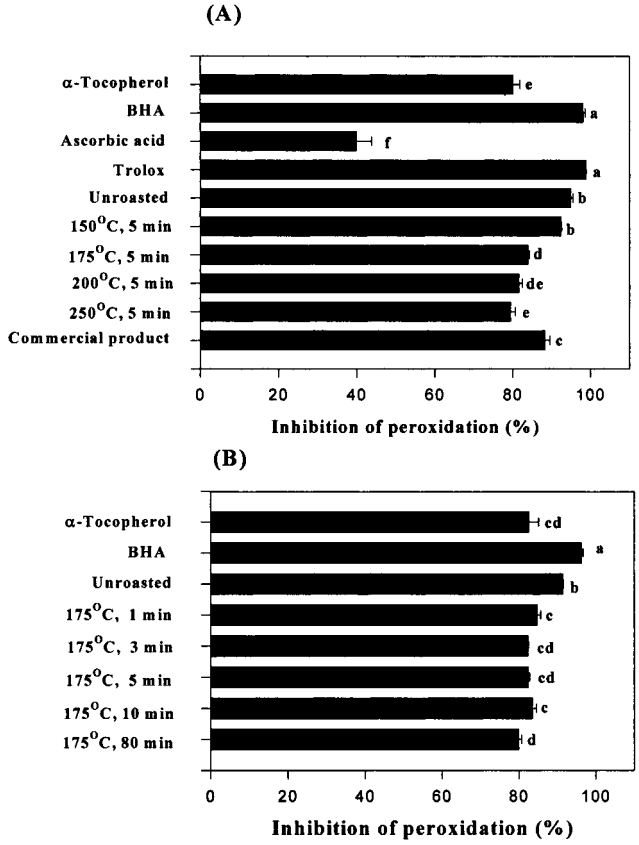

Figure 1. Antioxidant activities of water extracts from *C. tora* L. (0.2 mg/mL) prepared under different degrees of roasting. [a–f]Each value is the mean ± standard deviation of three replicate analyses. Values in each line with different letters are significantly different ($P<0.05$).

in Figure 1, the extracts of unroasted *C. tora* exhibited good antioxidant activity in the linoleic acid peroxidation system. At a concentration of 0.2 mg/mL, the extracts of unroasted *C. tora* inhibited 94% peroxidation of linoleic acid after incubation for 72 h. This percentage was higher than that of α-tocopherol (82%) and ascorbic acid (41%) but lower than that of BHA (98%) and Trolox (99%). Traditionally, the commercial product of *C. tora* has been roasted in order to enhance the flavor during infusion. Therefore, to evaluate the effect of different degrees of roasting on the antioxidant activity, the *C. tora* was roasted at different roasting temperatures. As shown in Figure 1A, the inhibition of peroxidation of linoleic acid were in the order of unroasted (94%) = roasted at 150 °C (92%) > commercial product (88%) > roasted at 175 °C (85%) > roasted at 200 °C (82%) >

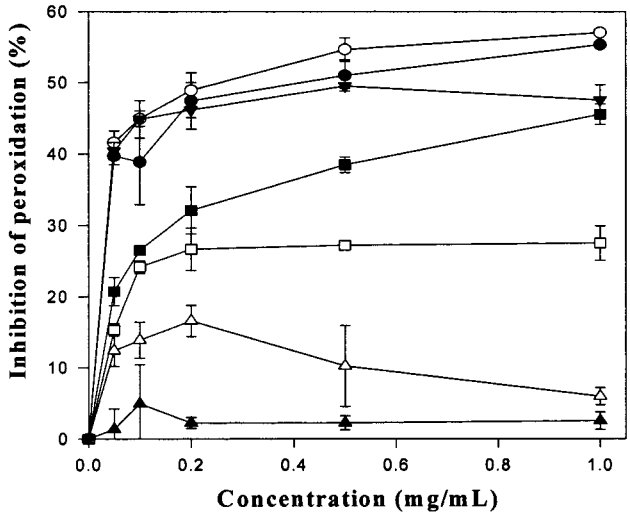

Figure 2. Effects of water extracts from *C. tora* L. prepared under different degrees of roasting on the lipid peroxidation of liposomes induced by Fe^{3+}/H_2O_2/ascorbic acid: −●−, unroasted; −○−, 150 °C, 5 min; −■−, 200 °C, 5 min; −□−, 250 °C, 5 min; −▲−, mannitol; −△−, EDTA; −▼−, α-tocopherol.

roasted at 250 °C (79%). The antioxidant activity of extracts of *C. tora* was significantly ($P < 0.05$) decreased with an increase in roasting temperature, except for the unroasted and those roasted at 150 °C. The commercial product of *C. tora* exhibited a stronger antioxidant activity than those roasted at 200 °C, which might be due to the commercial product with the addition of other Chinese herbs during preparation.

There were no differences ($P > 0.05$) in antioxidant activity when the *C. tora* was roasted at 175 °C for 1−10 min, but there was a decrease when it was roasted for 80 min (Figure 1-B). Nicoli et al. (1997) evaluated the effect of the degree of roasting on the antioxidant activity of coffee brews. They found that the antioxidant activity of coffee brews increased with roasting time from 0 to 10 min and then gradually decreased after 10 min of roasting. They also indicated that the increase in antioxidant activity of coffee brews under minimum roasting was due to the formation of Maillard reaction products and their phenolics content. However, the phenolics in coffee was degraded after over-roasting and caused the decrease in antioxidant activity. These results are different from those in the present study. The reason might be due to the difference in composition and degree of browning after roasting between coffee and *C. tora*. During the roasting process, the roasting time is the major factor to influencing the color appearance of *C. tora*. The color appearance of *C. tora* when roasted at 200 °C for 5 min is similar to that of commercial products. Therefore, the antioxidant activity of *C. tora* when roasted at 150, 200, and 250 °C for 5 min was compared in the following study.

Effect of WECT on Liposomes Peroxidation Induced by Fe^{3+}/H_2O_2/Ascorbic Acid. The inhibition of WECT on peroxidation of the cell membrane was evaluated using liposomes induced by Fe^{3+}/H_2O_2/ascorbic acid. As shown in Figure 2, the samples of unroasted *C. tora* and those roasted at 150 °C had better antioxidant activity. They exhibited about 40% inhibition effect at a concentration of 0.05 mg/mL, then increased to 56% at a concentration of 1 mg/mL that was higher than that of α-tocopherol (47%). Although the antioxidant activity of extracts of *C. tora* roasted at 200 and 250 °C was weaker than that of the unroasted,

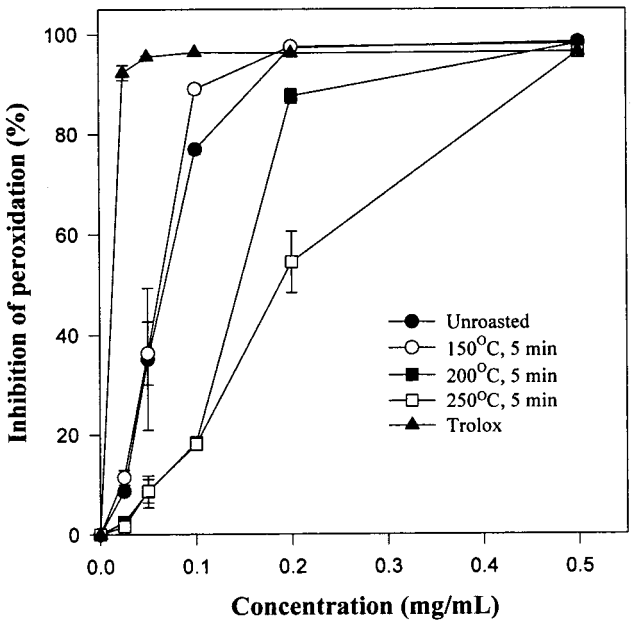

Figure 3. Effects of water extracts from *C. tora* L. prepared under different roasting degrees on the enzymatic lipid peroxidation of microsomes induced by NADPH/ADP/Fe^{3+}.

those roasted at 150 °C and α-tocopherol, it was higher than that of EDTA and mannitol. In this study, the antioxidant activity of all the test samples was in the order of unroasted = roasted at 150 °C > α-tocopherol > roasted at 200 °C > roasted at 250 °C > EDTA > mannitol at a concentration of 0.5 mg/mL. This result is similar to the trend of inhibition of peroxidation of linoleic acid (Figure 1).

Halliwell and Gutteridge (1989) indicated that free radical scavengers do not inhibit the peroxidation of some membrane lipids (liposome or microsome) induced by Fenton reaction. Damage to the membrane might result from the "site-specific" effect caused by direct binding of iron ion with the membrane. In the present study, mannitol showed no inhibition effect while EDTA showed a weak scavenging effect because it cheleted the iron ion on the membrane. Saija et al. (1995) demonstrated that quercetin has better antioxidant activity than rutin in a biomembrane system. The reason may be that quercetin rather than rutin interacting with that bilayer membrane of phospholipid. Therefore, the antioxidant activity of antioxidants in a membrane system depends on their ability not only to donate a hydrogen atom but also to incorporate into the membrane. α-Tocopherol scavenge the peroxyl radicals formed from the lipid peroxidation in the inner membrane (Ratty et al., 1988). Therefore, it can be predicted that there are some water-insoluble components in the unroasted samples and those roasted at 150 °C which can be incorporated into the membrane to afford antioxidant activity.

Effect of WECT on Enzymatic Lipid Peroxidation of Microsomes Induced by NADH/ADP/Fe^{3+}. Figure 3 shows the effects of extracts from *C. tora* prepared under different degrees of roasting on enzymatic lipid peroxidation of rat liver microsomes induced by NADH/ADP/Fe^{3+}. In this study, the antioxidant activity of all the samples of WECT increased in a concentration dependent manner. The inhibitory effects of all the samples were in the order of unroasted (97.5%) = roasted at 150 °C (97.2%) > roasted at 200 °C (87.6%) > roasted at 250 °C (54.4%) at a concentration of 0.2 mg/mL. Trolox exhibited an inhibitory effect of over 90% at a concentration lower than 0.05 mg/mL, but it showed a similar inhibitory effect at a concentration greater than 0.05 mg/mL. The antioxidant activity of extracts from unroasted *C. tora* and those roasted at 150 °C was equal to that of Trolox at a concentration greater than 0.2 mg/mL. Malterud et al. (1993) indicated that anthraquinones and anthrone inhibit the formation of thiobarbituric reactive substances in rat liver cell induced by *tert*-butyl hydroperoxide. Since anthraquinones are major pharmacological compounds of *C. tora* (Duke, 1992), they may have an antioxidant effect in this microsomes peroxidation system.

Table 2. Change in the Browning Intensity of Water Extracts from *C. tora* L. Prepared under Different Degrees of Roasting

roasting conditions	browning[a]
unroasted	0.246[a,b]
150 °C, 5 min	0.397[b]
200 °C, 5 min	0.778[d]
250 °C, 5 min	0.448[c]

[a] Browning intensity was measured the absorbance at 420 nm. [b] Values in a column with different superscripts are significantly different ($P < 0.05$).

Browning Intensity. Maillard reaction products have been reported to possess scavenging activity on reactive oxygen species (Hayase et al., 1990; Yen and Hsieh, 1995). The browning of extracts from *C. tora* with different degrees of roasting was determined in order to reveal its relation with antioxidant activity. As shown in Table 2, the browning of extracts significantly increased ($P < 0.05$) with an increase in roasting temperature up to 200 °C, but it decreased slightly at 250 °C of roasting. In general, browning increased with increasing reaction time; however, it might be decreased at a longer reaction time owing to the formation of nonsoluble compounds of high molecular weight (Lingnert and Ericksson, 1987). Thus, the absorbance may have decreased at a roasting temperature of 250 °C because of over-roasting, which led to polymerization of Maillard reaction products.

Yen and Hsieh (1995) indicated that the antioxidant activity of Maillard reaction products increased with an increase in reaction time. In the present study, the antioxidant activity of WECT decreased with an increase in degree of roasting. Nicoli et al. (1997) reported that the antioxidant activity of water extracts of coffee increased with an increase in roasting time of up to 10 min. However, the antioxidant activity of coffee extracts decreased with longer roasting time. They suggested that this might be due to the degradation of antioxidative Maillard products under over-roasting. Such can also explain the decrease in the antioxidant activity of WECT under over-roasting.

Chemiluminescence Intensity. Many chemical reactions produce chemiluminescence due to energy release energy, such as in the case of degradation of hydroperoxide, activated macrophage, Fenton reaction, and a singlet oxygen changing from excited state to ground state. (Halliwell and Gutteridge, 1989; Sato et al., 1991). Namiki et al. (1993) indicated that the Maillard reaction also produces chemiluminescence. As shown in Table 2, the browning of water extracts of *C. tora* increased after roasting. Thus, the chemiluminescence of WECT was determined (Figure 4).

Figure 4 shows that unroasted *C. tora* had very weak chemiluminescence; however, the intensity of chemiluminescence of *C. tora* increased with an increasing

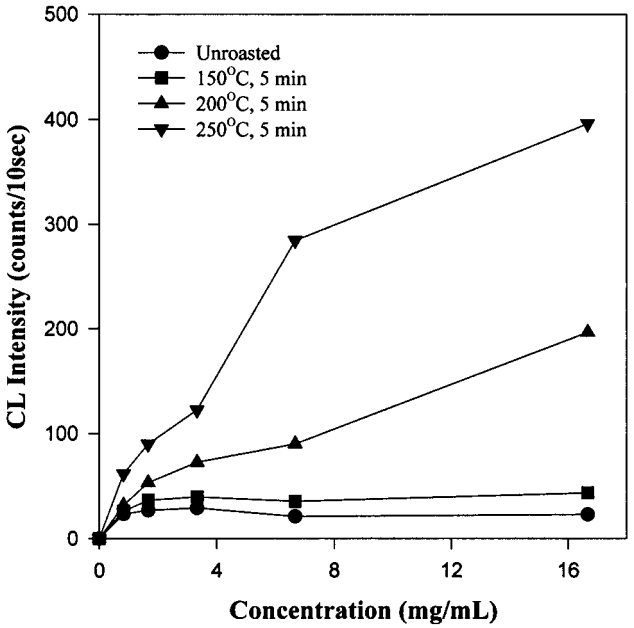

Figure 4. Chemiluminescence (CL) intensity of water extracts from *C. tora* L. prepared under different degrees of roasting.

Table 3. Changes in Free Amino Acids of Water Extracts from *C. tora* L. (WECT) with Different Degrees of Roasting

mg/g sample[a]	unroasted	150 °C[b]	200 °C	250 °C
aspartic acid	1.368	0.870	0.236	*c*
glutamic acid	1.517	0.310	0.019	
serine	0.480	0.248	0.037	
histidine	0.310	0.037	0.007	
glycine	0.529	0.247	0.055	0.020
threonine	0.438	0.145	0.021	
arginine	0.440	0.356		
alanine	0.902	0.496	0.057	0.023
tyrosine	0.368	0.068	0.065	0.042
methionine	0.153	0.021	0.015	
valine	0.649	0.301		
phenylanine	0.381	0.140		
isolucine	0.412	0.129		
leucine	0.410	0.113	0.013	
lysine	0.436	0.122	0.018	
total	8.793	3.602	0.543	0.085

[a] Contents of amino acid in WECT was expressed by amino acid (mg)/WECT (g). [b] The roasting time was 5 min. [c] Not detected.

degree of roasting. The intensity of the chemiluminescence of *C. tora* was on the order of roasted at 250 °C (396.0 counts/10 s) > roasted at 200 °C (196.2 counts/10 s) > roasted at 150 °C (43.3 counts/10 s) > unroasted (22.8 counts/10 s) at a concentration of 16.7 mg/mL. The chemiluminescence produced in the Maillard reaction might come from the free-radical products or low molecular weight hydroperoxides, such as dicarbonyl and pyrazinium compounds (Namiki et al., 1993). In the present study, the change in chemiluminescence intensity of WECT correlated with its degree of browning. Therefore, it can be said that roasting *C. tora* increased in browning and produced some free radical products or hydroperoxides, which resulted in the decrease in antioxidant activity.

Changes in Free Amino Acids. The degree of browning roasted *C. tora* increased due to the Maillard reaction during the roasting process. Table 3 shows the changes in the free amino acids of WECT with different degrees of roasting. The free amino acids of WECT decreased from 8.79 mg/g to 0.09 mg/g after roasting at 250 °C. Arginine, valine, phenylalanine, and isoleucine in WECT disappeared when roasting at 200 °C, while only glycine, alanine, and tyrosine remained after roasting at 250 °C. Jayalekshmy and Mathew (1990) reported that the total amino acids of coconut, especially lysine, arginine, and serine, decreased during the roasting process. The decrease of free amino acids in water extracts of roasted *C. tora* revealed the involvement of the Maillard reaction. Since the amount of amino acids also affects antioxidant activity, the decrease of free amino acids in water extracts of roasted *C. tora* is related to the decrease in antioxidant activity.

Table 4. Total Phenolic Compounds Contents of Water Extracts from *C. tora* L. (WECT) Prepared under Different Degrees of Roasting

roasting conditions	contents[a] (mg/g)
unroasted	$180.64 \pm 6.51^{a\,b}$
150 °C, 5 min	164.72 ± 6.43^{b}
200 °C, 5 min	121.95 ± 3.27^{c}
250 °C, 5 min	103.55 ± 3.08^{d}

[a] Contents of polyphenolic compounds in WECT was expressed by total phenolic compound (mg)/WECT (g). [b] Values in a column with different superscripts are significantly different ($P < 0.05$).

Total Polyphenols in Extracts from *C. tora* with Different Degrees of Roasting. Tsushida et al. (1994) reported that the antioxidant activity of vegetable extracts is related to their polyphenol content. Baldio (1996) indicated that the higher content of polyphenols in olive oil increased the oxidative stability of olive oil as determined by the Rancidmat method. *C. tora* contains many phenolic compounds, such as emodin, rhein, chrysophanol, and obtusin, some of which belong to anthraquinones (Duke, 1992). Some anthraquinones have been reported to have antioxidant activity (Malterud et al., 1993). As shown in Table 4, the total polyphenols in extracts of *C. tora* decreased significantly ($P < 0.05$) during roasting. The amount of polyphenols in extracts of *C. tora* decreased from 180.64 to 103.55 mg/g after roasting at 250 °C. This means that polyphenols were degraded during the roasting process. The decrease of polyphenols contents in *C. tora* after roasting is correlated the decrease in antioxidant activity. Therefore, it can be suggested that the decrease in antioxidant activity of extracts of roasted *C. tora* was related to the decrease in polyphenols.

CONCLUSIONS

This study shows that the extracts of unroasted *C. tora* had greater inhibition effect on peroxidation of linoleic acid than that of α-tocopherol. The extracts from *C. tora* roasted at 170 °C for 80 min and at 200 °C for 5 min showed equal inhibition effects on peroxidation of linoleic acid. This means that the antioxidant activity of *C. tora* was reduced by higher roasting temperature and longer roasting periods. The extracts of unroasted *C. tora* also exhibited good antioxidant activity in the liposome peroxidation system induced by Fenton reaction as well as in the enzymatic microsome peroxidation system. Therefore, the oxidation of biological membrane in vivo may be inhibited. The antioxidant activity of extracts from roasted *C. tora* decreased as compared with that of unroasted sample. This results might be caused by several factors which include (1) the reduction of phenolics in *C. tora* as a result of roasting and (2) the degradation of Maillard reaction products under

over-roasting making them oxidation products without antioxidant activity.

LITERATURE CITED

Baldioli, M.; Servili, M.; Perretti, G.; Monedoro, G. F. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *J. Am. Oil. Chem. Soc.* **1996**, *37*, 1589−1593.

Benavente-Garcisa, O.; Castillo, J.; Marin, F. R.; Ortuno, A.; Rio, J. A. D. Use and properties of citrus flavonoids. *J. Agric. Food Chem.* **1997**, *45*, 4505−4515.

Chen, M.-F.; Mo, L.-R.; Lin, R.-C.; Kuo, J.-Y.; Chang, K.-K.; Liao, C.; Lu. F.-J. Increase of resting levels of superoxide anion in the whole blood of patients with decompensated liver cirrhosis. *Free Rad. Biol. Med.* **1997**, *23*, 672−679.

Duke, J. A. *Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants*; CRC Press: Boca Raton, FL, 1992; pp 143−144.

Halliwell, B.; Gutteridge, J. M. C. In *Free Radicals in Biology and Medicine*; Halliwell, B., Gutteridge, J. M. C., Eds.; Clarendon Press: Oxford, 1989a.

Halliwell, B.; Gutteridge, J. M. C. Free radicals and toxicology. In *Free Radicals in Biology and Medicine*; Halliwell, B., Gutteridge, J. M. C., Eds.; Clarendon Press: Oxford, 1989b; pp 299−365.

Hanna, A. N.; Sharma, H. M.; Kauffman, E. M.; Newwan, H. A. I. In vitro and in vivo inhibition of microsomal lipid peroxidation by MA-631. *Pharmacol. Biochem. Behav.* **1994**, *39*, 505−510.

Hayase, F.; Hirashima, S.; Okamato, G.; Kato, H. Scavenging of active oxygens by melanoidins. *Agric. Biol. Chem.* **1990**, *54*, 855−862.

Hertog, M. G. L.; Hollman, P. C. H. Potential health effects of the dietary flavonoid quercetin. *Eur. J. Clin. Nutr.* **1996**, *50*, 63−66.

Hertog, M. G. L.; Feskeens, E. J. M.; Hollman, C. H.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: de Zutphen elderly. *Lancet* **1993**, *342*, 1007−1011.

Jayalekshmy, A.; Mathew, A. D. Changes in the carbohydrates and proteins of coconut during roasting. *Food Chem.* **1990**, *37*, 123−134.

Kim, S. Y.; Kim, J. H.; Kim, S. K.; Oh, M. J.; Jung, M. Y. Antioxidant activities of selected oriental herb extracts. *J. Am. Oil Chem. Soc.* **1994**, *71*, 633−640.

Lingnert, H.; Ericksson, C. E. Antioxidative effect of maillard reaction products. *Prog. Fd. Nutri. Sci.* **1981**, *5*, 453−466.

Malterud, K. E.; Farbrot, T. L.; Huse, A. E.; Sund, R. B. Antioxidant and radical scavenging effects of anthraquinones and anthrones. *Pharmacology* **1993,** *47*(Suppl. 1), 77−85.

Namiki, M.; Oka, M.; Otsuka, M.; Miyazawa, T.; Fujimoto1, K.; Namiki, K. Weak chemiluminescence at early stage of the Maillard reaction. *J. Agric. Food Chem.* **1993**, *41*, 1704−1709.

Nicoli, M. C.; Anese, M.; Manzocco, L.; Lerici, C. R. Antioxidant properties of coffee brews in relation to the roasting degree. *Lebensm.-Wiss. U.-Technol.* **1997**, *30*, 292−297.

Ratty, A. K.; Sunamoto, J.; Das, N. P. Interaction of flavonoids with 1,1-diphenyl-2-picrydrazyl free radical, liposomal membranes and soybean lipoxygenase-1. *Biochem. Pharmocol.* **1988**, *37*, 989−995.

Saija, A.; Scalese, M.; Lanza, M.; Marzullo, D.; Bonina, F.; Castelli, F. Flavonoids as antioxidant agents: importance of their interaction with biomembranes. *Free Radical Bio. Med.* **1995**, *19*, 481−486.

Salah, N.; Miller, N. J.; Paganga, G.; Tijburg, L.; Bolwell, G. P.; Rice-Evans, C. Polyphenolic flavonols as scavenger of aqueous phase radicals and as chain-breaking antioxidants. *Arch. Biochem. Biophys.* **1995**, *2*, 339−346.

SAS Institute, Inc. *SAS User's Guide: Statistics*; SAS Institute, Inc.: Cary, NC, 1985.

Sato, T.; Inaba, H.; Kawai, K.; Furukawa, H.; Hirono, I.; Miyazawa. T. Low-level chemiluminescence from drosophila melanogaster fed with chemical mutagens polycyclic aromatic hydrocarbon quinones and a carcinogenic bracken fern. *Mutat. Res.* **1991**, *251*, 91−97.

Stampfer, M. J.; Henneekens, C. H.; Manson, J. E.; Colditz, G. A.; Rosner, B.; Willet, W. C. Vitamin E consumption and the risk of coronary disease in women. *N. Engl. J. Med.* **1993**, *328*, 1444−1449.

Su, J.-D. Investigation of antioxidative activity and tocopherol contents on Chinese crude drugs of fruits and seeds. *Food Sci. (Chinese)* ***1992***, *19*, 12−24.

Taga, M. S.; Miller, E. E.; Pratt, D. E. Chia seeds as a source of natural lipid antioxidants. *J. Am. Oil Chem. Soc.* **1984**, *61*, 928−931.

Tsuda, T.; Ohshima, K.; Kawakishi, S.; Osawa, T. Antioxidative pigments isolated from the seeds of *Phaseolus vulgaris* L. *J. Agric. Food Chem.* **1994**, *42*, 248−251.

Tsushida, T.; Suzuki, M.; Kurogi, M. Evaluation of antioxidant activity of vegetable extracts and determination of some active compounds. *Nippon Shokuhin Kogyo Gakkaishi.* **1994**, *41*, 611−618.

Yen, G. C.; Chen, H. Y. Comparison of antimutagenic effect of various tea extracts (green, oolong, pouchong, and black tea). *J. Food Prot.* **1994**, *57*, 54−58.

Yen, G. C.; Hsieh, P. P. Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products. *J. Sci. Food. Agric.* **1995**, *67*, 415−420.

Yen, G. C.; Hsieh, C. L. Antioxidant activity of extracts from Du-zhong (*Eucommia ulmoides*) toward various lipid peroxidation models in vitro. *J. Agric. Food Chem.* **1998**, *46*, 3952−3957.

Yen, G. C.; Chen, H. W.; Duh, P. D. Extraction and identification of an antioxidative component from Jue Ming Zi (*Cassia tora* L.). *J. Agric. Food Chem.* **1998**, *46*, 820−824.

Zhang, Q.; Yin, J.; Zhang, J. Comparison of contents of some active components between crude and processed seeds of sickle senna (*Cassia tora*) and their detections by HPLC. *Chinese Herb (in Chinese)* **1996**, *27*, 79−81.

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