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Total Phenolics and Antioxidant Activities of Fenugreek, Green Tea, Black Tea, Grape Seed, Ginger, Rosemary, Gotu Kola, and Ginkgo Extracts, Vitamin E, and *tert*-Butylhydroquinone

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The total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and *tert*-butylhydroquinone, were determined. Grape seed and green tea were analyzed for their phenolic constituents using high-performance liquid chromatography. The total phenolics of the plant extracts, determined by the Folin−Ciocalteu method, ranged from 24.8 to 92.5 mg of chlorogenic acid equivalent/g dry material. The antioxidant activities of methanolic extracts determined by conjugated diene measurement of methyl linoleate were 3.4−86.3%. The antioxidant activity of the extracts using chicken fat by an oxidative stability instrument (4.6−10.2 h of induction time) followed a similar trend in antioxidant activity as determined by the Folin−Ciocalteu method. Seven phenolics in grape seed and green tea extracts were identified that ranged from 15.38 to 1158.49 and 18.3 to 1087.02 mg/100 g of extract, respectively. Plant extracts such as green tea and grape seed extracts can be used to retard lipid oxidation in a variety of food products.

KEYWORDS: Antioxidant activities; phenolics; induction time; chlorogenic acid; linoleate

INTRODUCTION

Antioxidants can minimize or prevent lipid oxidation in food products (*1*). Synthetic antioxidants such as *tert*-butylhydroxytoluene, *tert*-butylhydroxyanisole, and *tert*-butylhydroquinone (TBHQ) have been widely used to retard lipid oxidation in foods (*2*). However, such synthetic antioxidants are not preferred due to toxicological concerns. For this reason, there have been increasing interests in identifying plant extracts to minimize/retard lipid oxidation in lipid-based food products (*3*). Most of these natural antioxidants come from fruits, vegetables, spices, grains, and herbs (*4*). Green tea contains 50% flavonoids such as catechin and epicatechin that contribute to antioxidant activities (*5*−*8*). Polymeric tannins and monomeric flavonoids such as catechin and epicatechin in grape seed extracts could be responsible for their higher antioxidant activities (*9, 10*). Rosemary has been shown to have antioxidant activity in ground pork products (*11*). The antioxidant activities in rosemary extracts could be due to carnosol, carnosic acid, rosmarinic acid, rosmanol, and rosemaridiphenol phenolic compounds (*12*). Vitamin E and TBHQ function as oxygen scavengers in fat-containing foods to prevent lipid oxidation (*13*). Ginkgo has been reported to have strong antioxidant activities due to flavone glycosides that scavenge free radicals (*14*). Gingerol, gingerdiol, gingerdione, and other compounds could be responsible for antioxidant activities of ginger (*15, 16*). The oxidative stability instrument (OSI) (*17*) and conjugated diene (*18*) are two main methods used to evaluate antioxidant activities.

It is hoped that information on the total phenolics and antioxidant activities of plant extracts and their individual phenolic compounds can be used as criteria to retard or prevent lipid oxidation in a variety of food products. The objectives of this study were to (i) evaluate green tea, black tea, fenugreek, grape seed, gotu kola, rosemary, ginger, and ginkgo extracts, vitamin E, and TBHQ for total phenolics and antioxidant activities and (ii) select plant extracts that demonstrated higher antioxidant activities in objective i and identify phenolics by high-performance liquid chroamtography (HPLC).

MATERIALS AND METHODS

Materials. Green tea and black tea (Celestial Seasoning, Inc., Boulder, CO) were purchased from a local supermarket. Gotu kola and ginkgo extracts, ginger powder, and fenugreek (*Trigonella foe-mumgraecum*) seeds were purchased from Avalon Foods (Fayetteville, AR). Commercial grape seed extract powder was obtained from Mega Natural Inc. (Madera, CA). TBHQ (Tenox 20A, contains 20% TBHQ) was obtained from EaHPLCstman Chemical Company (Kingsport, TN). Rosemary and vitamin E were obtained from Danisco Cultor (United States). The chicken fat was obtained from a local company (Tyson, Fayetteville, AR). The methyl linoleate was purchased from VWR Scientific Products Inc. (Fayetteville, AR). The standard phenolics were purchased from Sigma Chemical Co. (St. Louis, MO).

Fenugreek Extract Preparation. The fenugreek extract was prepared by an established procedure in our laboratory. Fenugreek (*T. foenumgraecum*) seeds were milled in a Cyclotec mill (Cyclotec 1093

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Sample Mill, Tecator AB Box 70, Höganas, Sweden) and passed through 60 mesh. The ground seeds were defatted in hexane (1:4) with agitation (T-Line lab stirrer, Talboys Engineering Corp., Emerson, NJ) at setting 40 for 30 min and centrifuged (IEC, CRU-5000, Internal Equipment Company, Needham Heights, MA) at 2500*g* for 10 min at 25 °C. The residue was defatted again using the same procedure. The defatted residue was air-dried overnight under a fume hood to remove the residual hexane. The defatted residue was extracted with 60% ethyl alcohol (1:3) with stirring for 30 min and filtered under vacuum. The filtrate was concentrated in a Rotavapor (Buchi 011, Buchi, Switzerland) and dried under a fume hood overnight. The dried samples were freeze-dried at <100 mTorr vacuum (Virtis Freeze-Dryer SQ25LE, the Virtis Company, Gardiner, NY). The fenugreek extracts were stored at 4 °C until they were used. The same procedure (preparation of fenugreek extract) was conducted for the preparation of the ginger extract.

Green and Black Tea Extracts Preparation. The same procedure was followed for the preparation of extract for both green tea and black tea. The tea was ground in a coffee grinder (Braun Aromatic KSM2, Braun Canada Div., Gillette Canada Company, Mexique) for 1 min. The ground tea powder was mixed with water (1:10) and boiled for 10 min. The supernatant was recovered through vacuum filtration. The filtrate was then frozen to −20 °C and freeze-dried at <100 mTorr vacuum. The resulting extract was stored at 4 °C until it was used.

Determination of Total Phenolics. Fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and TBHQ were used for the determination of total phenolics by the Folin−Ciocalteu method (*19*). Fifty milligrams of each extract was weighed into 50 mL plastic extraction tubes and vortexed with 25 mL of the extraction solvent (40 mL acetone:40 mL methanol:20 mL water: 0.1 mL formic acid). Then, the sample with the extraction solvent was heated at 60 °C (water bath) for 1 h, allowed to cool to room temperature, and homogenized for 30 s with a sonicator at setting 6 (Virtishear Tempest, The Virtis Co.). The homogenized sample was filtered through Micracloth into a screw-capped test tube.

The filtrates from each extract (200 μL, three replicates) were introduced into screw cap test tubes; 1.0 mL of Folin−Ciocalteu's reagent and 1.0 mL of sodium carbonate (7.5%) were added. The tubes were vortexed and allowed to stand for 2 h. The absorption at 726 nm was measured (Perkin-Elmer λ15 UV−vis spectrophotometer, Norwalk, CT). The total phenolic content was expressed as chlorogenic acid equivalents (CAE) in milligrams per gram dry material.

$$\text{total phenolics concentration (mg/g)} = (A/b) \times [(\text{SW} + 25)/\text{SW}]$$

where A = absorbance at 726 nm, SW = sample weight (g), and b = slope of the standard curve of chlorogenic acid.

Separation and Quantification of Phenolics by HPLC. Chromatographic equipment consisting of a Hewlett-Packard (Avondale, PA) liquid chromatograph model 1090 equipped with a diode array UV detector and a TSK-GEL Super-ODS (Supelco, Bellefonte, PA) column was used. At absorbances of 254 and 234 nm, the effluent was monitored. The mobile phase consisted of solvents A, B, and C. Solvent A was 0.1% trifluoroacetic acid in acetonitrile, solvent B was 0.1% trifluoroacetic acid in HPLC grade water, and solvent C was 100% methanol. The flow rate was set at 1.0 mL/min, and the column temperature was maintained at 37 °C throughout the test. The initial solvent condition was 0% solvent A and 100% solvent B. A linear gradient was used to increase solvent A from 0 to 10% within 7 min. Solvent A was then increased from 10 to 40% using a 20 min linear gradient. This composition was then maintained for 2 min and returned to the initial condition in 3 min. Solvent C was used for column washing after each run (*20*).

One hundred milligrams of grape seed and green tea extracts was dissolved in 25 mL of methanol and passed through a 0.2 μm filter before analysis by HPLC. The sample size of 4 μL for each methanolic extract was injected during HPLC analysis. The identification and concentration of phenolics in grape seed and green tea were calculated from standard curves calibrated using phenolic standards. The phenolic contents were expressed as milligrams per 100 g of each plant extract.

Antioxidant Activity Determination−Methyl Linoleate. The antioxidant testing was carried out by oxidizing linoleic acid methyl ester (MeLo) in the presence of antioxidants as described by Heinonen et al. (*18*). Ten milligrams of each extract was dissolved in 50 mL of methanol. After that, 0.5 mL from each methanolic extract was added to MeLo (0.2 g), and methanol was evaporated under nitrogen. Sample aliquots (10 mg) were taken at zero time and after 72 h at 40 °C in the dark and dissolved in 5 mL of 2,2,4-trimethylpentane (isooctane), and the conjugated diene absorption at 234 nm was measured using a spectrophotometer (Perkin-Elmer λ15 UV−vis spectrophotometer). The antioxidant activity was expressed as percentage (%) inhibition of formation of MeLo-conjugated diene hydroperoxides after 72 h of oxidation as compared with a blank from MeLo. The percentage inhibition of linoleic acid oxidation was calculated as follows:

$$\%\ \text{inhibition} = [(AB_{72h} - AB_{0h}) - (AE_{72h} - AE_{0h})/(AB_{72h} - AB_{0h})] \times 100$$

where A = absorbance, E = extract, and B = blank.

Antioxidant Activities of Plant Extracts Using Chicken Fat Substrate by OSI. The purpose of this experiment was to investigate the effect of plant extracts that have the highest antioxidant activities to prevent lipid oxidation in chicken fat. Chicken fat was used because it consists of a complex system of triglycerides with different types of fatty acids, phospholipids, and other components (*21*).

The oxidative stability of the plant extracts and their combinations at three levels (500, 3000, and 6000 ppm), TBHQ (200 ppm), and the control (commercial chicken fat, local industry in Arkansas) were evaluated using an OSI (Omnion Inc., Rockland, MA). Triplicate samples were sonicated for 30 s in ice with the melted chicken fat (melted in a water bath at 60 °C) with a sonicator at setting 6 (Virtishear Tempest, The Virtis Co.) and transferred into the OSI tubes. The sample tubes were held in a thermostatic block in the OSI at 110 °C, and a stream of air at 210 g/cm^2 was bubbled through the sample. The air pressure was allowed to equilibrate at 176 g/cm^2. The volatiles released from the sample passed through rubber tubing into a tube containing deionized water and a conductivity probe. A water trap was placed between the sample tube and the conductivity tube to facilitate the condensation of water from the sample and to aid the free flow of volatiles into the conductivity tube. The probe measured the change in conductivity of deionized water due to collected volatiles. The induction period was the time, in hours, before detectable levels of volatile organic acids were trapped in the deionized water. The software analyzed the data and generated an induction period as the OSI number. A longer induction period indicated a better oxidative stability of the sample.

Statistical Analysis. Data were presented as means of three determinations and analyzed using the general linear model procedure with SAS Version 8.2 software package (*22*). Least significant difference analysis was used to compare means. Significant differences were defined at $p < 0.05$.

RESULTS AND DISCUSSIONS

Amount of Total Phenolics. The amounts of total phenolics varied widely in fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts and ranged from 24.8 to 92.5 mg CAE/g dry material (**Table 1**), while the values for vitamin E and TBHQ (that were used as controls) were 142.1 and 196.1 mg CAE/g dry material, respectively. This variation is expected in plant extracts due to other constituents as well as the variation in the type of phenolics. Among plant extracts, rosemary contained a relatively higher amount of phenolics (92.5 mg CAE/g), whereas lower levels were found in ginkgo, gotu kola, and ginger (24.8, 25.4, and 39.8, respectively). The amounts of phenolics in green tea, black tea, and grape seed extracts were very similar (59.8, 59.3, and 63.5 mg CAE/g, respectively). Plant extracts contain a range of phenolic compounds and derivatives including simple phenolics such as benzoic acid, tannins, lignans, lignins, stilbenes, and flavonoids and long-chain carboxylic acids (complex structure) such as cutin and suberin (*12*).

Table 1. Total Phenolics (mg of CAE/g dw)[a] and Antioxidant Activities by Conjugated Diene Method (%)[a] of Plant Extracts, Vitamin E, and TBHQ

sample	total phenolics (mg of CAE/g dw)	antioxidant activities (%)
green tea extract	59.8 ± 1.8 d*	70.1 ± 2.4 c*
black tea extract	59.3 ± 0.3 d	52.0 ± 1.7 d
grape seed extract	63.5 ± 1.0 d	86.3 ± 1.1 b
ginger extract	39.9 ± 2.6 f	6.0 ± 0.4 f,g
ginkgo extract	24.8 ± 1.4 g	3.4 ± 0.3 g
gotu kola extract	25.4 ± 1.2 g	3.8 ± 0.2 g
rosemary extract	92.5 ± 1.8 c	39.1 ± 1.2 e
fenugreek	54.3 ± 2.6 e	10.0 ± 0.7 f
vitamin E	142.1 ± 6.7 b	90.3 ± 2.0 b
TBHQ	196.1 ± 3.4 a	97.7 ± 1.8 a

[a] All values are on a dry basis. The values are the means of three determinations. *Column values with the same letters were not significantly different ($p < 0.05$).

Table 2. Phenolic Contents of Grape Seed and Green Tea Extracts (mg/100 g Extracts)[a]

phenolics	plant extracts	
	green tea	grape seed
protocatechuic	22.67 ± 1.4	93.7 ± 3.6
caffeic acid	830.1 ± 18.4	33.79 ± 1.5
gentistic acid	ND[b]	472.78 ± 18.4
vanillic acid	61.17 ± 4.0	ND
syringic acid	75.91 ± 3.2	253.42 ± 7.1
catechin	18.3 ± 1.3	887.44 ± 28.7
epicatechin	1087.02 ± 44.4	1158.49 ± 51.2
benzoic acid	319.78 ± 11.9	ND
gallic acid	ND	15.38 ± 1.3

[a] All values are on a dry basis. The values are means of three determinations. [b] Not detected.

Antioxidant Activities Determination—Methyl Linoleate. The antioxidant activities of plant extracts in MeLo are given in **Table 1**. The antioxidant acitivities of ginger, ginkgo, and gotu kola extracts were negligible (ranged from 3.4 to 6.0%). A higher level of antioxidant activities was observed in green tea and grape seed extracts (70.1 and 86.3%, respectively). The antioxidant activities of vitamin E and TBHQ were 90.3 and 97.7%, respectively. Although green tea and grape seed extracts contained a lower phenolic content than vitamin E and TBHQ, proportionally higher antioxidant activities demonstrated that the type of phenolics rather than the amounts is responsible for antioxidant activities. These results agreed with Kahkonen et al. (*23*) and Shahidi et al. (*12*), who reported that differences in antioxidant activities of plant extracts could be due to different structures of plant extracts from phenolic acids and flavonoids compounds as well as their derivatives. For instance, the antioxidant activities of phenolic acids and their derivatives such as esters depend on the number of hydroxy groups in the molecules. The higher antioxidant activities in green tea and grape seed extracts could be due to higher amounts of caffeic acid and epicatechin in green tea extracts and epicatechin and catechin in grape seed extracts (**Table 2**). These results agreed with Namiki (*5*) and Alan and Miller (*24*) who reported that the superior activity of green tea leaves could be due to catchins and epicatechin and their gallic acid esters. Peng et al. (*10*) reported that the contents of grape seed from polymeric and monomeric (catechin and epicatechin) types could be responsible for the higher antioxidant activities. Grape seed and green tea extracts with higher antioxidant activities were selected from several plant extracts for further evaluation in poultry products.

Table 3. Antioxidant Activities by Conjugated Diene Method (%)[a] of Green Tea and Grape Seed Extracts and Combinations

treatment	antioxidant activities (%)
grape seed extract (GSE) 1000 ppm	89.2 ± 1.3
GSE 500 ppm	85.8 ± 2.2
GSE 200 ppm	74.2 ± 1.6
green tea extract (GTE) 1000 ppm	88.8 ± 1.4
GTE 500 ppm	70.1 ± 3.6
GTE 200 ppm	41.0 ± 5.2
GTE + GSE 1000 ppm	90.7 ± 1.4
GTE + GSE 500 ppm	84.4 ± 1.4
GTE + GSE 200 ppm	66.6 ± 2.0

[a] All values are on a dry basis. The values are the means of three determinations.

Table 4. Antioxidant Activities of Grape Seed and Green Tea Extracts and TBHQ Using Chicken Fat Substrate by OSI[a]

sample	level (ppm)	induction time (h)
grape seed extract	6000	9.9 ± 0.2 b*
grape seed extract	3000	6.2 ± 0.1 d
grape seed extract	500	4.8 ± 0.1 f
green tea extract	6000	7.6 ± 0.2 c
green tea extract	3000	5.6 ± 0.1 e
green tea extract	500	4.6 ± 0.1 f
green tea + grape seed	6000	10.1 ± 0.2 b
green tea + grape seed	3000	6.6 ± 0.2 d
green tea + grape seed	500	4.6 ± 0.1 f
TBHQ	200	39.9 ± 1.1 a
control		2.3 ± 0.1 g

[a] All values are on a dry basis. The values are the means of three determinations. *Column values with the same letters were not significantly different ($p < 0.05$).

Separation and Quantification of Phenolics by HPLC. The phenolic acids in grape seed and green tea extracts determined by HPLC are given in **Table 2**. The phenolic contents in grape seed and green tea extracts ranged from 15.38 to 1158.49 and 18.3 to 1087.02 mg/100 g extract, respectively. Caffeic acid (830.10 mg/100 g extract) and epicatechin (1087.02 mg/100 g extract) were the main phenolics in green tea, while in grape seed extracts epicatechin (1158.49 mg/100 g extract), catechin (887.44 mg/100 g extract), and gentistic acid (472.78 mg/100 g extract) were the major phenoics. These results supported the findings by Fadhel and Amran (*8*) and Balentine et al. (*6*), who reported that the main flavonoids in green tea are catechins and epicatechin. Peng et al. (*10*) reported that 55% of grape seed procyanidins consists of polymeric and monomeric types such as catechin and epicatechin.

Antioxidant Activities Determination—Methyl Linoleate of Green Tea and Grape Seed Extracts at Different Levels and Combinations. The antioxidant activities of green tea and grape seed extracts and combinations increased with an increase in concentration (200, 500, and 1000 ppm) in the linoleate model system (**Table 3**) and ranged from 41 to 88.8, 74.2 to 89.2, and 41 to 91.7%, respectively. Different types of phenolics, including different compounds and condensed phenolics, could have contributed to the above observations of Kahkonen et al. (*23*) and Shahidi and Marian (*12*).

Antioxidant Activities of Green Tea and Grape Seed Extracts at Different Levels and Combinations Using Chicken Fat Substrate by OSI. In the linoleate model system, green tea and grape seed extracts demonstrated the highest antioxidant activities. Hence, these two extracts separately and in combination were evaluated for antioxidant activities in a chicken lipid system. The induction times of plant extracts in lipid oxidation were much lower than TBHQ (39.9 h) and ranged from 4.6 to

10.2 h (**Table 4**) but were higher than that of the control (2.3 h). No significant differences were observed at 500 ppm ($p < 0.05$) between single and combined extracts, while at 3000 and 6000 ppm grape seed and combined extracts (grape seed and green tea extracts) were higher than green tea extracts [6.2, 6.6, and 5.6 (at 3000 ppm) and 9.9, 10.1, and 7.6 induction time (h) (at 6000 ppm), respectively]. A lower antioxidant activity in chicken fat was detected in green tea extracts. The antioxidant activity determination by the methyl linoleate method was useful to screen the best plant extracts that have the highest antioxidant activities, and OSI is a versatile instrument to monitor the effect of these extracts on the oxidative stability of chicken fat.

In conclusion, total phenolic contents differed among plant extracts. The type of phenolics contributed to antioxidant activities. The high antioxidant activities in green tea and grape seed extracts could be due to higher amounts of caffeic acid and epicatechin in green tea extracts and epicatechin and catechin in grape seed extracts. MeLo and OSI systems can be used effectively to determine antioxidant activities in model and food systems. Green tea and grape seed extracts show potentials to retard lipid oxidation in a variety of food products.

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