JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Added Caffeic Acid and Tyrosol on the Fatty Acid and Volatile Profiles of Camellia Oil following Heating

ZHONG HAIYAN,[†] DANNY R. BEDGOOD JR.,[‡] ANDREA G. BISHOP,[‡] PAUL D. PRENZLER,[‡] AND KEVIN ROBARDS*,[‡]

Faculty of Life Science and Technology, Central South Forestry University, Changsha 410004, Hunan, People's Republic of China, and School of Science and Technology, Charles Sturt University, Locked Bag 588, Wagga Wagga 2678, Australia

Camellia oil is widely used in some parts of the world partly because of its high oxidative stability. The effect of heating a refined camellia oil for 1 h at 120 °C or 2 h at 170 °C with exogenous antioxidant, namely, caffeic acid and tyrosol, was studied. Parameters used to assess the effect of heating were peroxide and *K* values, volatile formation, and fatty acid profile. Of these, volatile formation was the most sensitive index of change as seen in the number of volatiles and the total area count of volatiles in gas chromatograms. Hexanal was generally the dominant volatile in treated and untreated samples with a concentration of 2.13 and 5.34 mg kg^{-1} in untreated oils heated at 120 and 170 °C, respectively. The hexanal content was significantly reduced in heated oils to which tyrosol and/or caffeic acid had been added. Using volatile formation as an index of oxidation, tyrosol was the more effective antioxidant of these compounds. This is contradictory to generally accepted antioxidant structure−activity relationships. Changes in fatty acid profiles after heating for up to 24 h at 180 °C were not significant.

KEYWORDS: Camellia oil; caffeic acid; fatty acid; heating; oxidative stability; tyrosol; volatile

INTRODUCTION

Plant oils provide a rich source of polyunsaturated fatty acids. These compounds are preferred targets of free radical-induced oxidation although endogenous antioxidants afford some protection to the oils. High temperatures such as those encountered in commercial frying accelerate the degradation of oils. Oxidation and the effects of high temperature on oil stability have been studied extensively from theoretical (*1*, *2*) and practical perspectives. The latter studies (*3*−*5*) have involved the use of a variety of oils under conditions including exposure to air in the presence and absence of light, room temperature, and high-temperature storage and under frying conditions (*6*). Such studies demonstrate the importance of oil composition (*7*, *8*) and oxygen concentration (*9*). Barrera-Arellano et al. (*10*) showed that the expected influence of the degree of fatty acid unsaturation was evident only when oils were unprotected by antioxidants or possessed identical initial antioxidant contents. The choice of indices used to assess the extent of deterioration is also a critical factor (*11*).

The role of endogenous and added antioxidants in improving thermal oxidative stability has also been investigated. It can be concluded that the major lipophilic antioxidants, tocopherols, provide limited protective action (*7*, *12*) that is supported by the polar phenolic compounds in the oils (*13*, *14*). Tocopherols were lost very rapidly during heating (*10*), and this may account for their limited protective action at elevated temperatures. Tocopherols may provide protection at ambient temperature, but the phenols nevertheless contribute significantly to the oxidative stability of cold-pressed oils (*15*). The improved thermal stability of soybean and rice-bran oils modified with tree-seed oils (*16*) can be attributed to the presence of phenolic antioxidants in the tree-seed oils.

The present study examines the thermal stability of a nontraditional tree-seed oil. Oil-tea camellia has the largest plantation area and the highest annual oil yield of all of the woody oil plants in China. Camellia oil has a special taste and flavor, good storage stability, and positive health effects (*17*) as well as superior oxidative stability relative to other seed oils, such as soybean and corn oil. This can be attributed to both high oleic acid and low polyunsaturated fatty acid content and also to the level of phospholipids. Crude camellia oil was observed to be more stable than refined and supercritical CO_2 extracted oil (*18*), which may be related to natural phenolic content. The addition of α-tocopherol to camellia oil significantly increased the induction time (*18*). However, the effect of phenols on camellia oil at high temperatures (such as during cooking) is poorly understood. In this study, the effect of added caffeic acid and tyrosol on the oxidative stability of refined camellia oil is investigated. Indicators used are peroxide value (PV) and *K* values, volatile profiles, and fatty acid profiles

* To whom correspondence should be addressed. Tel: +61-2-6933 2540. Fax: +61-2-6933 2737. E-mail: krobards@csu.edu.au.
[†] Central South Forestry University.
[‡] Charles Sturt University.

10.1021/jf061974z CCC: $33.50
Published on Web 11/18/2006

during heating at temperatures from 120 to 170 °C. There are health concerns associated with the consumption of trans-fatty acids (*19*−*23*), and the isomeric distribution of fatty acids was also investigated as were changes in phenolic composition during heating by analyses of total phenols and phenolic profile. This is the first multidimensional study addressing the thermal oxidative stability of camellia oil.

MATERIALS AND METHODS

Materials. Expressed refined camellia oil was provided by Soyatech International Ltd. (Queensland, Australia). Weighed quantities of caffeic acid and tyrosol were dissolved in methanol/chloroform (15 mL; 1:2 v/v) to obtain the desired phenolic concentration (50, 100, or 150 mg kg^{-1}) when added to 150 g of oil. The phenolic compounds were mixed with oil for 20 min in the dark (*24*), and the organic solvent was stripped by bubbling nitrogen for 6 h, after which constant mass was achieved. Treated and untreated oils (40 g) were placed in uncapped glass vials (25 mL capacity) and heated in a fan circulated oven at 120 and 170 °C. A more limited range of measurements was also performed at 180 °C, but these did not differ significantly from the data for 170 °C. Samples were withdrawn at various time intervals up to 24 h, analyzed, and discarded. All treatments and analyses were performed in at least duplicate.

Standards. Pentanal, undecan-1-ol (Merck, Germany), hexanal, heptanal, octanal, pent-1-ene-3-ol, pentanol, octanol (Aldrich, United States), octanal, (Sigma, United States), and cyclooctanol (Ajax) were used for obtaining retention data.

Fatty Acid Profile. The fatty acid profile was determined as fatty acid methyl esters by gas chromatography (Analytical Services, New South Wales Department of Primary Industry). Methanoic potassium hydroxide (0.2 mL; 0.2 M) was added to the oil (0.1 g) in *n*-heptane (2 mL). The mixture was shaken vigorously and allowed to stand and separate. The heptane phase was removed and dissolved in a further 1 mLof *n*-heptane. Separation of the fatty acid esters was performed on a Varian 3800 Gas Chromatograph with a Supelco SP 2340 column (60 m × 0.25 mm i.d.; 0.2 μm film). The column temperature was programmed at 165 °C for 10 min and then increased to 200 °C at 2 °C/min with a final isothermal period of 13 min. Helium was used as carrier gas with constant flow at 1.2 mL min^{-1}. The injector temperature was set at 245 °C with a split ratio of 1:50. The FID detector temperature was 245 °C. Data were analyzed using Star Workstation Chromatography software (version 4.51). Quantification was performed by external calibration. Relative errors were less than 1% at all concentration levels.

Total Phenols. Oil (3 g) was dissolved in hexane (15 mL) and extracted with aqueous methanol (3 × 5 mL; 50:50 v/v) by shaking for 2 min for each extraction. The sample was left to stand overnight. An aliquot (1 mL) was transferred to a volumetric flask (10 mL) to which was added Folin-Ciocalteu reagent (0.5 mL). The solution was shaken and left to stand for 3 min prior to addition of saturated (*ca.* 10%) sodium carbonate solution (1 mL) and dilution to volume with water. After 1 h, absorbance at 725 nm was measured against a reagent blank using a Cary 50 spectrophotometer. Calibration was performed using caffeic acid in the range 0 to 100 μg/ 10 mL. The calibration curve was $y = 124.3x + 0.080$; $R^2 = 0.9988$.

Phenol Profile. Oil (15 g) was dissolved in hexane (15 mL) and extracted three times with methanol:water (1 mL; 50:50 v/v). The aqueous phase was then washed with hexane (3 × 3 mL). The hexane was discarded and the aqueous phase was filtered through a 0.45 μm nonsterile, plastic filter prior to analysis. HPLC was performed using a Varian 9012 pump equipped with a 20 μL sample loop injector. Separation was achieved on a Phenomenex C18 column (150 mm × 4.6 mm; 5 μm) with gradient elution at a mobile phase flow rate of 1 mL min^{-1}. The binary solvent mixture comprised solvent A (water: acetic acid, 100:1 v/v) and solvent B (methanol:acetonitrile:acetic acid, 95:5:1 v/v). The LC gradient was ramped linearly from 10% solvent B to 30% solvent B in 10 min, then 5 min isocratic, then a further linear ramp to 40% solvent B over 10 min, then from 40 to 50% solvent B in 15 min, to 100% solvent B in 10 min, 5 min isocratic, and returned to initial conditions over 10 min. The column eluent was monitored at 278, 259, and 240 nm with a Varian 9065 Polychrom detector.

Volatile Profile. Volatile compounds from the oils were analyzed by solid-phase microextraction-gas chromatography (SPME-GC). Volatiles were extracted using a polydimethylsiloxane/carbowax/divinylbenzene fiber (PDMS/CAR/DVB, 50/30 μm, Supelco) as follows: Oil (1 g) was placed in a sealed 10 mL reactivial and equilibrated at 40 °C for 15 min. The SPME fiber was inserted through the septum and left in the headspace at the same temperature for 30 min. The fiber was retracted and immediately transferred into the GC, where it was desorbed for 1 min using a splitless injection port at 250 °C. A Varian Star 3400 gas chromatograph with a SGE BPX5 column (30 m × 0.25 mm i.d.; 0.25 μm film) was used. The column was initially held at 40 °C for 8 min, increased at 5 °C/min to 200 °C with a final isothermal period of 10 min. The flow rate of nitrogen carrier gas was 2 mL min^{-1}. Chromatograms were routinely monitored by an FID detector, which was maintained at 300 °C. The concentration of individual volatiles was expressed as hexanal. For identification, volatiles were analyzed by gas chromatography−mass spectrometry by thermal desorption in the injection port of a Varian 3400X gas chromatograph (Melbourne, Australia) coupled with a Saturn 2000 ion trap mass spectrometer using the same chromatographic conditions as above. The electron impact ionization (EI) mode with automatic gain control (AGC) was used for MS. The electron multiplier voltage for MS was 1850 V, AGC target was 25000 counts, and filament emission current was 15 μA with axial modulation amplitude at 4.0 V. The ion trap temperature was maintained at 250 °C, and the manifold temperature was at 60 °C. The temperature of the transfer line, interfacing the GC and MS, was set at 250 °C. Mass spectral scan time from *m*/*z* 35 to 450 was 0.8 s (using 2 microscans). Background mass was set at 45 *m*/*z*.

Spectrophotometric Analysis. Clear refined camellia oil (0.25 g) was accurately weighed, dissolved, and filled to the mark with spectrograde cyclohexane in a volumetric flask (25 mL). The camellia oil solution was homogenized and where opalescence or turbidity was observed, the solution was discarded and a fresh perfectly clear solution was prepared. Absorbance was measured at appropriate wavelengths with a spectrophotometer (Cary 50 UV−vis Spectrophotometer, Varian, Melbourne, Australia) and 1 cm quartz cells using the spectro-grade cyclohexane as a reference. The *K* values were calculated from $K_\lambda = A_\lambda/(c\ l)$ where K_λ = specific extinction at wavelength, λ; A_λ= absorbance measured at wavelength, λ (e.g., 232 nm, 270 nm); c = concentration of the solution in g/100 mL; and l = cuvette thickness (cm).

Statistical Analysis. Data were analyzed using SPSS 11.5 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Three heating temperatures were investigated to produce different levels of oil deterioration since heating temperature has been established as an important variable in the oxidation of edible oils (*25*). Fruhwirth et al. (*13*) found that the higher the content of endogenous, hydrophilic phenolic compounds, the higher the oxidative stability in a range of oils including olive oil which has a similar fatty acid profile to camellia oil. The refined camellia oil used in this study was chosen for its low endogenous phenolic content of 4.1 ± 0.7 μg g^{-1} (Folin-Ciocalteu total phenols measured at 725 nm, expressed as caffeic acid) (*26*). Caffeic acid and tyrosol were selected as exogenous phenolic antioxidants as neither occurred naturally in the camellia oil. Tyrosol reportedly has a low antioxidant capacity as compared to caffeic acid, which is the subject of numerous, ongoing oxidation studies in different contexts (*27*−*32*). These compounds have the common structural feature of a single aromatic ring.

Effect of Heating on Peroxide and *K* Values. The refined camellia oil had no off-odors and an initial PV of 14.9 meq kg^{-1}. Heating of the oil at 120 °C for 1 h caused an increase in

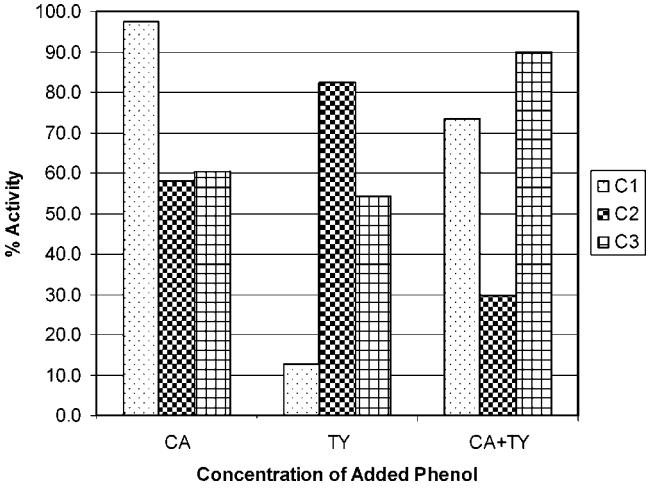

Figure 1. Antioxidant activity in refined camellia oil heated at 120 °C for 1 h. C1, C2, and C3 refer to the concentrations of added antioxidant as 50, 100, or 150 mg kg^{-1}, and CA and TY refer to caffeic acid and tyrosol, respectively. The standard error is 4%.

Table 1. *K* Values of Refined Camellia Oil before and after Heating at 120 °C for 1 h or 170 °C for 2 h

	120 °C		170 °C	
	K_{232}	K_{270}	K_{232}	K_{270}
before heating	3.91	1.38	3.91	1.38
after heating	4.55	1.38	3.77	1.95

PV to 17.3 meq kg^{-1} while the PV decreased to 3.6 meq kg^{-1} after heating of the oil at 170 °C for 2 h indicating a net formation of hydroperoxides at the lower temperature and a net destruction at the higher temperature. Addition of the phenolic antioxidants to the camellia oil reduced the percentage change in PV when the oil was heated at 120 °C. This inhibition can be expressed as an antioxidant activity calculated as:

$$\% \text{ activity} = \Delta PV_0 - \Delta PV_{phenol}/\Delta PV_0 \times 100\%$$

where ΔPV_0 = change in PV of control oil, i.e., no added phenol and ΔPV_{phenol} = change in PV for oil with added phenol.

Data for antioxidant activity at 120 °C are shown in **Figure 1**. When the oil was heated at 170 or 180 °C for 2 h (data not shown), there was a reduction in PV. This reduction is probably a function of the short heating time destroying existing peroxides. Low initial rates of oxidation were also reported (*33*) for the thermal oxidation of olive oils at 100 °C. The reduction in PV was enhanced by addition to the oil of caffeic acid and tyrosol alone and in admixture (data not shown). At both temperatures, there was no clear trend between activity and the nature of the antioxidant or its concentration.

K values also exhibited temperature dependence (**Table 1**). At 120 °C, the addition of caffeic acid or tyrosol, alone or in admixture (data not shown), reduced the extent of increase in K_{232}, relative to untreated oil. At 170 °C, antioxidant addition enhanced the decrease in K_{232} seen in untreated oil. At 120 °C, heating of the oil containing added antioxidant produced no change in K_{270}. However, there was an enhanced increase in K_{270} in oils containing added antioxidant after heating at 170 °C. Since K_{232} provides an indicator of the content of diene conjugated bonds, while K_{270} reflects triene concentration, it appears that diene conjugation was destabilized by antioxidant addition at 120 °C but stabilized at 170 °C. In contrast, triene formation was unaffected at 120 °C by antioxidant addition but was enhanced at 170 °C.

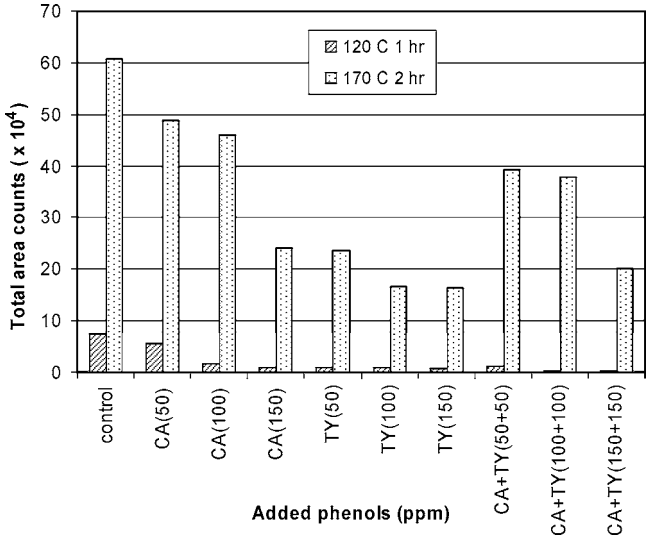

Figure 2. Effect of added phenols on the volatile content of refined camellia oil measured before and after heating at 120 and 170 °C. Data are expressed as total area counts of volatiles eluting between 2.4 and 50.0 min. Using this *y*-scale, volatiles before heating are "0".

Overall, the addition of caffeic acid generally resulted in greater protection of the oil than addition of tyrosol but with no simple concentration effect observed under these experimental conditions. No real synergistic effect was observed for caffeic acid and tyrosol in admixture. The greater activity of caffeic acid relative to tyrosol is consistent with its *o*-diphenolic structure, which is traditionally associated with high antioxidant activity (*34*, *35*). These results reflect the data of Naz et al. (*6*) who used PV to follow the relative rates of oxidative deterioration in edible oils fried at 180 °C for 30−90 min. However, the level of antioxidant added was 3 orders of magnitude higher than in the current study.

Effect of Heating on Volatile Profile. The formation of volatile compounds as secondary products of lipid oxidation provides a further measure of the extent of camellia oil decomposition associated with heating. Volatile formation was monitored using SPME-GC and is evaluated here first by considering the total area counts for chromatographic peaks with retention times between 2.4 and 50.0 min, as an indicator of overall volatile formation. **Figure 2** presents this data for a range of spiked oil samples before and after heating at 120 and 170 °C. Total area count of volatiles provided a more sensitive and predictable indicator of oil deterioration than simple measures of oxidation such as PV.

Before heating, the oil samples produced only a small number of volatiles that were detected at low concentrations by this method. However, after heating a range of volatiles emerged with increased concentrations. The total area counts in samples heated at 120 °C ranged from a maximum around 7.5×10^4 in the control down to 2.6×10^3 for the sample spiked with caffeic acid plus tyrosol (150 mg kg^{-1} of each) in admixture. The effect of adding increasing concentrations of caffeic acid was to decrease volatile formation. Addition of tyrosol resulted in even greater suppression of volatile formation; however, the most marked decrease in volatiles at this heating temperature was evident in the samples spiked with both phenols.

Some similarities were observed in volatile formation after heating at 170 °C for 2 h. The maximum in total area counts, 6.1×10^5 was again observed for the untreated camellia oil.

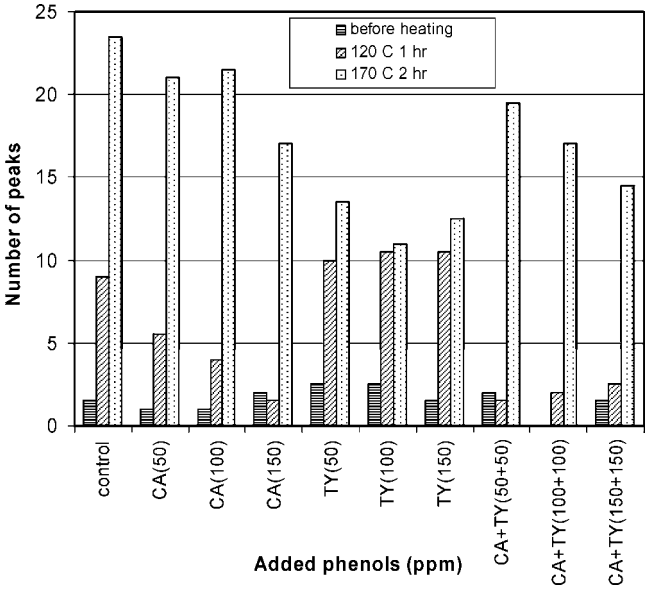

Figure 3. Effect of added phenols on the volatile content of camellia oil measured before and after heating at 120 and 170 °C. Data are expressed as the number of volatile peaks eluting between 2.4 and 50.0 min with a concentration exceeding 0.02 mg kg^{-1} as hexanal.

Increased concentrations of caffeic acid resulted in decreased volatile formation. The effect was once again more pronounced with the addition of tyrosol, to a minimum total area count of 1.6×10^5 for the sample spiked with 150 mg kg^{-1} of tyrosol. Interestingly, the effect of adding both phenols approached an average of the two individual compounds. This indicates that the presence of caffeic acid may even inhibit the effectiveness of tyrosol as an antioxidant in this system. Pinelo et al. (*36*) observed a negative synergism between phenolic antioxidant compounds in ethanolic solutions.

Figure 3 expands the understanding of volatile formation in these samples, presenting the number of peaks observed in the gas chromatograms with retention times between 2.4 and 50.0 min. Prior to heating of nonspiked and spiked samples, SPME-GC of the refined oil samples detected up to three volatile peaks at concentrations exceeding 0.02 mg kg^{-1} as hexanal. This number increased after heating at 120 °C and again after heating at 170 °C when between 10 and 24 peaks were typically detected. The largest number of volatiles was evident in the nonspiked control and a general trend of decreasing number of peaks was observed with increasing concentration of added phenols. After heating at the higher temperature, tyrosol was observed to be a more effective inhibitor of oxidation than caffeic acid. Again, the effect of combining caffeic acid and tyrosol markedly diminished the antioxidant efficiency of tyrosol, with the number of peaks lying between that observed for additions of the individual phenols.

Considering both sets of data (**Figures 2** and **3**) together creates a clearer picture of volatile formation. Heating at 120 °C with added tyrosol produced around 10 peaks, but with relatively low area counts, compared to oils spiked with caffeic acid which produced fewer (*2*−*6*) peaks but with significantly higher total area counts; for example, by as much as a factor of 6 in the case of 50 mg kg^{-1} spikes. Heating samples with added tyrosol at 170 °C produced only about two additional peaks but significantly higher area counts. After the same treatment, samples containing caffeic acid produced around 17−22 peaks with total area counts 1.5−2 times that of the samples treated with the same concentrations of tyrosol. Hence, tyrosol appears to act more effectively as an antioxidant in these cases than caffeic acid. When the two phenols are added in combination to the camellia oil samples, fewer peaks are observed in the chromatograms than for either single phenol; however, the areas of these peaks were relatively high.

Identification of the Major Volatile Compounds. The major volatiles present in the oil following heating were identified by a combination of retention data and mass spectral fragmentation. **Table 2** details the changing concentrations of volatile compounds that eluted between 2.4 and 50.0 min. The only peaks detected at a concentration exceeding 0.02 mg kg^{-1} as hexanal in the unheated nonspiked control sample were cyclooctanol (0.70 mg kg^{-1}) and pentanal (0.27 mg kg^{-1}). The major peaks in the control sample after heating at 120 °C were (in order of decreasing concentration): hexanal, pentanal, *E*-2-heptenal, cyclooctanol, penten-3-ol, and heptenal. For samples spiked with caffeic acid, the major volatiles again in order of decreasing concentration were hexanal, cyclooctanol, and *E*-2-heptenal; while for samples containing tyrosol they were hexanal, *E*-2-heptenal, cyclooctanol, octanal, and pentanal. Heating samples containing both caffeic acid and tyrosol at 120 °C produced only two detectable volatiles with retention times in this region: hexanal and cyclooctanol.

The major peaks evident in the control sample after heating at 170 °C for 2 h were (in order of decreasing concentration): 1-octene, hexanal, penten-3-ol, cyclooctanol, *E*-2-heptenal, two isomers of 2,4-decadienal, pentanol, pentanal, octanal, heptanal, and undecanol. These compounds also were present after heating the spiked samples, although at lower concentrations with caffeic acid and even lower with tyrosol. In samples treated with tyrosol (100 or 150 mg kg^{-1}), the concentrations of pentanal, pentanol, and heptanal fell below the detection limit. Heating samples containing both caffeic acid and tyrosol at 170 °C produced the same suite of volatiles at concentrations between those for oils treated with the single phenols. The five most concentrated compounds were 1-octene, hexanal, cyclooctanol, penten-3-ol, *E*-2-heptenal, and the later eluting isomer of 2,4-decadienal (t_R = 26.3).

The volatile profile can be related to the fatty acid content of the oil. Thus, the absence of hexenals from the volatile profile is consistent with the trace levels of linolenic acid in camellia oil. On the other hand, the presence of the various saturated aldehydes in heated samples can be accounted for by breakdown of various hydroperoxides produced by thermo-oxidation of fatty acids. For example, hexanal was produced by breakdown of linoleate 13-hydroperoxide; *E*-2-heptanal by decomposition of linoleate 12-hydroperoxide, pentanal and heptanal by decomposition of linoleate 13/11-hydroperoxide, and octanal from oleate 11-hydroperoxide (*37*). Hexanal was a dominant volatile observed to increase in concentration with heating temperature (**Table 2**). The highest concentrations were evident in the heated nonspiked controls with varying concentrations in the heated oils to which antioxidant was added. Temperature dependence and concentration trends were similar for the remaining volatiles in **Table 2**. In all cases, tyrosol was more effective than caffeic acid in inhibiting the formation of volatile aldehydes. Thus, on the basis of inhibition of volatile formation, tyrosol was a more effective antioxidant than caffeic acid. This is contrary to general theories of antioxidant activity and ideas about structure−activity relationships, which associate enhanced antioxidant activity with an increase in the number of phenolic hydroxyl groups. However, we have previously shown (*38*) that tyrosol was an effective antioxidant in a lipid system consistent with the current

Table 2. Effect of Added Phenols on Refined Camellia Oil Volatiles (mg kg^{-1} as Hexanal) following Heating at 120 °C for 1 h or 170 °C for 2 h

	compound (retention time, min)[a]											
sample	penten-3-ol (2.44)	pentanal (2.57)	pentanol (3.93)	1-octene (4.65)	hexanal (5.03)	heptanal (10.56)	*E*-2-heptenal (13.43)	octanal (15.42)	cyclooctanol (19.33)	undecanol (24.55)	2,4-decadienal (25.57)	2,4-decadienal (26.30)
120 °C, no addition	0.14	0.73	ND[b]	ND	2.13	0.13	0.42	ND	0.19	ND	ND	ND
caffeic acid, 50 mg kg^{-1}	ND	0.38	ND	ND	1.61	ND	0.40	ND	0.42	ND	ND	ND
caffeic acid, 100 mg kg^{-1}	ND	ND	ND	ND	0.82	ND	0.13	ND	0.13	ND	ND	ND
caffeic acid, 150 mg kg^{-1}	ND	ND	ND	ND	0.33	ND	ND	ND	0.15	ND	ND	ND
tyrosol, 50 mg kg^{-1}	ND	0.04	ND	ND	0.24	ND	0.11	0.03	0.09	ND	ND	ND
tyrosol, 100 mg kg^{-1}	ND	ND	ND	ND	0.17	ND	0.12	0.03	0.16	ND	ND	ND
tyrosol, 150 mg kg^{-1}	ND	ND	ND	ND	0.09	ND	0.08	0.03	0.17	ND	ND	ND
caffeic acid + tyrosol, 50 + 50 mg kg^{-1}	ND	ND	ND	ND	0.36	ND	ND	ND	0.16	ND	ND	ND
caffeic acid + tyrosol, 100 + 100 mg kg^{-1}	ND	ND	ND	ND	0.16	ND	ND	ND	ND	ND	ND	ND
caffeic acid + tyrosol, 150 + 150 mg kg^{-1}	ND	ND	ND	ND	0.13	ND	ND	ND	ND	ND	ND	ND
170 °C, no addition	3.60	0.88	0.90	9.29	5.34	0.67	1.87	0.73	2.31	0.57	0.95	1.68
caffeic acid, 50 mg kg^{-1}	2.93	0.60	0.79	7.82	4.47	0.64	1.64	0.63	1.98	0.43	0.71	1.21
caffeic acid, 100 mg kg^{-1}	2.34	0.46	0.66	6.91	4.30	0.68	1.89	0.72	2.21	0.47	0.88	1.45
caffeic acid, 150 mg kg^{-1}	1.28	0.13	0.13	2.84	1.83	0.39	1.05	0.48	1.41	0.34	0.71	1.24
tyrosol, 50 mg kg^{-1}	1.33	0.26	0.37	3.63	1.86	0.29	0.92	0.29	0.97	0.15	0.43	0.77
tyrosol, 100 mg kg^{-1}	1.73	ND	ND	2.09	1.18	ND	0.71	0.14	0.76	0.17	0.54	0.99
tyrosol, 150 mg kg^{-1}	0.75	ND	ND	2.18	1.32	0.13	0.87	0.31	0.80	0.27	0.50	0.83
caffeic acid + tyrosol, 50 + 50 mg kg^{-1}	1.81	0.51	0.60	5.99	3.48	0.54	1.56	0.64	2.05	0.46	0.74	1.34
caffeic acid + tyrosol, 100 + 100 mg kg^{-1}	1.87	0.55	0.61	5.48	3.51	0.52	1.30	0.52	1.82	0.45	0.86	1.53
caffeic acid + tyrosol, 150 + 150 mg kg^{-1}	1.22	ND	0.30	2.90	1.68	0.30	0.75	0.30	0.93	0.13	0.46	0.79

[a] All compounds were identified by reference to standards with the exception of the deca-2,4-dienals. [b] ND, not detected at 0.01 mg kg^{-1}.

observation. There was no evidence of synergistic effects for the two phenols, and the volatile levels in oils spiked with both phenols were generally approximated as an average of the data for individual additions.

Total Phenol and Phenol Profile. The Folin-Ciocalteu method was used to investigate the residual total phenol content after frying as in previous studies (*39*, *40*). Heating untreated camellia oil at 120 or 170 °C caused loss of 86 and 95%, respectively of the total endogenous phenols (4.1 ± 0.7 μg g^{-1}, measured by Folin-Ciocalteu method), while at 180 °C the endogenous phenols were entirely consumed. Oil samples spiked with 50 or 100 mg kg^{-1} of caffeic acid or tyrosol had their total phenols (endogenous plus added phenolics) reduced by around 40% after heating at 120 °C (**Table 3**). Samples containing 150 mg kg^{-1} of added phenol lost a smaller percentage of total phenols during heating at 120 °C: 17% for caffeic acid and 25% for tyrosol. At this temperature, spiking the camellia oil with a mixture of the two phenols resulted in losses in total phenols during heating that approximated an average for the two individual additives. Samples containing 50 or 100 mg kg^{-1} of caffeic acid, heated at the higher temperature lost 95−100% of the total phenols while with 150 mg kg^{-1} added, the loss fell to 79%. For tyrosol, the loss in total phenols decreased with concentration from 73 to 40%. With one exception (100 mg kg^{-1} caffeic acid + 100 mg kg^{-1} tyrosol), the amount of phenolic species lost (as measured by total phenols) decreased with concentration of added phenolic compound.

The classic Folin-Ciocalteu method is less specific and informative than the quantification of individual phenols by HPLC. Indeed, Chimi et al. (*29*) reported that phenolics were degraded as a consequence of their antioxidant activity and the rate of loss could be correlated to their antioxidant efficacy. The refined camellia oil used in this study exhibited no definable peaks in the HPLC chromatogram at 280 nm. There was no endogenous caffeic acid or tyrosol detected in the oil. The effect of heating on the concentrations of added caffeic acid and tyrosol as determined by HPLC analysis is presented in **Table 3**. At 120 °C, there was no detectable loss in caffeic acid or tyrosol when added to the camellia oil alone. When added in admixture, there was a concentration-dependent loss of caffeic acid with smaller loss of tyrosol at 150 mg kg^{-1} only. At the higher temperature, there was a concentration-dependent loss of caffeic acid when added alone and in admixture. Loss of tyrosol also occurred at this higher temperature but with no concentration dependence. These observations provide further support for the occurrence of different breakdown mechanisms at the two temperatures as suggested by the PV and *K* data.

Certainly, it would appear that the caffeic acid was consumed before the tyrosol, as 100, 98, and 91% of the caffeic acid was

Table 3. Effect of Heating on Loss of Folin-Ciocalteu Total Phenols and Caffeic Acid and Tyrosol from Refined Camellia Oil Heated at 120 °C for 1 h or 170 °C for 2 h

	% loss of phenol[a]					
	120 °C			170 °C		
sample treatment	total phenols	caffeic acid	tyrosol	total phenols	caffeic acid	tyrosol
caffeic acid, 50 mg kg^{-1}	40	0		94	100	
caffeic acid, 100 mg kg^{-1}	43	0		96	96	
caffeic acid, 150 mg kg^{-1}	17	0		79	79	
tyrosol, 50 mg kg^{-1}	42		0	73		55
tyrosol, 100 mg kg^{-1}	42		0	47		56
tyrosol, 150 mg kg^{-1}	25		0	40		52
caffeic acid + tyrosol, 50 + 50 mg kg^{-1}	26	25	0	76	100	52
caffeic acid + tyrosol, 100 + 100 mg kg^{-1}	43	33	0	68	98	55
caffeic acid + tyrosol, 150 + 150 mg kg^{-1}	25	50	10	64	91	58

[a] Data are expressed as means of triplicate determinations relative to unheated, spiked sample; the standard error is 3%. Total phenols are as measured by the Folin-Ciocalteu method; caffeic acid and tyrosol were measured by HPLC.

consumed as compared to 52, 55, and 58%, respectively, of the tyrosol as the additive concentration was increased. The preferential reaction of caffeic acid over tyrosol would account for at least one of the trends observed in volatile formation, namely, that caffeic acid reduced the antioxidant efficiency of tyrosol. Frying experiments at 180 °C using virgin olive oil and phenolic additives (*30*) showed a reduction in tyrosol concentration of 14% after 1 h of frying and of 20% after 2 h, from 6.59 to 5.26 mg kg^{-1}. Brenes et al. (*41*) simulated frying with virgin olive oils to examine changes in endogenous phenol content. Heating at 180 °C led to decreases in all phenolics, at rates dependent on the individual components. Tyrosol was found to decrease in concentration more slowly than other phenolics, and was not considered to contribute to the oils stability. Nissiotis and Tasioula-Margari (*33*) also noted tyrosol as a more stable and less protective phenolic in virgin olive oils during thermal oxidation at 60 and 100 °C. Pellegrini et al. (*39*) found that the antioxidant activity of tyrosol was significantly lower than that of caffeic acid in refined olive oil, consistent with the higher stability of hydroxyl radicals of the cinnamic acid derivative. It is notable that at the higher temperature (**Table 3**) approximately 50% of the tyrosol is used in the same time interval regardless of the initial concentration indicating that tyrosol decomposed according to first-order kinetics. This suggests that the process might involve thermo-oxidation while also exhibiting some antioxidant action (when volatiles are measured).

Fatty Acid Profile. Oxidation occurs via a complex series of reactions and is characterized by various changes in the oil, including a decrease in the total unsaturated fatty acid content of an oil. On a crude scale, we have considered these changes in terms of PV and *K* values and used volatile formation as a more sophisticated measure of oxidation products. We now turn attention to changes in concentrations of individual fatty acids as the reactants in the oxidation process. Twenty-five fatty acids ranging from C14 to C24 were resolved using the described FAME-GC method. The most prevalent fatty acid in the unheated camellia oil was oleic acid (C18:1; 720 mg g^{-1}), comprising around 84% of the fatty acid profile. The camellia oil contained 8−9% each of palmitic (C16:0; 74.3 mg g^{-1}) and linoleic (C18:2; 65.8 mg g^{-1}) acids, about 0.1% stearic acid (C18:0; 1.0 mg g^{-1}) and 0.01% linolenic acid (C18:3; 0.1 mg g^{-1}). The remaining fatty acids were detected at concentrations ranging from 0.01 to 0.1 mg g^{-1}. Unsaturated fatty acids were present as the *cis* isomers with only traces of the corresponding *trans* isomers (<0.02 mg g^{-1}). Heating the treated and untreated camellia oils at 180 °C (or at lower temperatures) for periods up to 24 h did not produce significant changes in the fatty acid profile. There was no evidence for *cis*−*trans* isomerization induced by heating of the oil. Murkovic and Pfannhauser (*7*) determined that the ratio of linoleic acid to oleic acid had significant influence on the oxidative stability of pumpkin seed oils, measured at 120 °C. Although extrapolation of stability data is risky, their results do suggest that camellia oil with the above oleic to linoleic acid ratio would be very stable. However, significant reduction in the percentage of both oleic and linoleic acids was observed after frying experiments on olive oil at 180 °C for 5−15 h (*4*). This is notable as the oleic acid and linoleic acid composition of the olive oil was very similar to that of the camellia oil.

This study has investigated the oxidation of oleic acid-rich camellia oil with a multidimensional approach by measuring PV, *K* values, volatile and phenolic compounds, and fatty acid profile. Measurement of volatile products of oxidation is seemingly able to detect changes in oils more reliably at an earlier stage of oxidation than measurement of the reactant species. This is probably a reflection of the ability of analytical techniques to detect formation of small quantities of new products vs detection of small changes in reactant concentrations. PV and *K* values were not sensitive indicators of changes induced by heating and did not show clear correlations with the addition of antioxidants caffeic acid and tyrosol. However, when volatile compounds were used as a measure of oxidative deterioration, clearer dose−antioxidant response trends could be identified. Surprisingly, using this measure of antioxidant activity, tyrosol proved to be the "better" antioxidant. However, when the phenolic compounds themselves were measured during the oil oxidation experiments, caffeic acid was lost faster than tyrosol. This more rapid loss of caffeic acid is not attributable to its antioxidant action. Higher concentrations of volatile compounds were found in oils with caffeic acid than in oils with tyrosol. Further work is required to establish the mechanism by which tyrosol acts to prevent volatile formation during oxidation of oils. Under the conditions in the present study, *cis* to *trans* isomerism was not observed for any of the fatty acids following heating.

ACKNOWLEDGMENT

The assistance of Curtis Kalua with chromatographic analyses is acknowledged.

LITERATURE CITED

(1) Leopoldini, M.; Marino, T.; Russo, N.; Toscano, M. Antioxidant properties of phenolic compounds: H-atom versus electron transfer mechanism. *J. Phys. Chem. A* **2004**, *108*, 4916−4922.

(2) Leopoldini, M.; Pitarch, I. P.; Russo, N.; Toscano, M. Structure, conformation, and electronic properties of apigenin, luteolin, and taxifolin antioxidants. A first principle theoretical study. *J. Phys. Chem. A* **2004**, *108*, 92−96.

(3) Quiles, J. L.; Ramirez-Tortosa, M. C.; Gomez, J. A.; Huertas, J. R.; Mataix, J. Role of vitamin E and phenolic compounds in the antioxidant capacity, measured by ESR, of virgin olive, olive and sunflower oils after frying. *Food Chem.* **2002**, *76*, 461−468.

(4) Velasco, J.; Marmesat, S.; Bordeaux, O.; Marquez-Ruiz, G.; Dobarganes, C. Formation and evolution of monoepoxy fatty acids in thermoxidized olive and sunflower oils and quantitation in used frying oils from restaurants and fried-food outlets. *J. Agric. Food Chem.* **2004**, *52*, 4438−4443.

(5) Gomez-Alonso, S.; Mancebo-Campos, V.; Salvador, M. D.; Fregapane, G. Oxidation kinetics in olive oil triacylglycerols under accelerated shelf-life testing (25−75 °C). *Eur. J. Lipid Sci. Technol.* **2004**, *106*, 369−375.

(6) Naz, S.; Sheikh, H.; Siddiqi, R.; Sayeed, S. A. Oxidative stability of olive, corn and soybean oil under different conditions. *Food Chem.* **2004**, *88*, 253−259.

(7) Murkovic, M.; Pfannhauser, W. Stability of pumpkin seed oil. *Eur. J. Lipid Sci. Technol.* **2000**, *102*, 607−611.

(8) Hoshina, R.; Endo, Y.; Fujimoto, K. Effect of triacylglycerol structures on the thermal oxidative stability of edible oil. *J. Am. Oil Chem. Soc.* **2004**, *81*, 461−465.

(9) Fujimoto, K. The effect of oxygen concentration on oxidative deterioration in frying oil. *Food Sci. Biotechnol.* **2003**, *12*, 694−699.

(10) Barrera-Arellano, D.; Ruiz-Mendez, V.; Velasco, J.; Marquez-Ruiz, G.; Dobarganes, C. Loss of tocopherols and formation of degradation compounds at frying temperatures in oils differing in degree of unsaturation and natural antioxidant content. *J. Sci. Food. Agric.* **2002**, *82*, 1696−1702.

(11) Shimizu, M.; Moriwaki, J.; Nishide, T.; Nakajima, Y. Thermal deterioration of diacylglycerol and triacylglycerol oils during deep-frying. *J. Am. Oil Chem. Soc.* **2004**, *81*, 571−576.

(12) Normand, L.; Eskin, N. A. M.; Przybylski, R. Comparison of the frying stability of regular and low-linolenic acid soybean oils. *J. Food Lipids* **2003**, *10*, 81−90.

(13) Fruhwirth, G. O.; Wenzl, T.; El-Toukhy, R.; Wagner, F. S.; Hermetter, A. Fluorescence screening of antioxidant capacity in a pumpkin seed oils and other natural oils. *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 266−274.

(14) Koski, A.; Pekkarinen, S.; Hopia, A.; Wahala, K.; Heinonen, M. Processing of rapeseed oil: Effects on sinapic acid derivative content and oxidative stability. *Eur. Food Res. Technol.* **2003**, *217*, 110−114.

(15) Koski, A.; Psomiadou, E.; Tsimidou, M.; Hopia, A.; Kefalas, P.; Wahala, K.; Heinonen, M. Oxidative stability and minor constituents of virgin olive oil and cold-pressed rapeseed oil. *Eur. Food Res. Technol.* **2002**, *214*, 294−298.

(16) Nasirullah. Development of deep frying edible vegetable oils. *J. Food Lipids* **2001**, *8*, 295−304.

(17) Zhong, H. Y.; Wan, C. N.; Xie, B. X. The present status and development tendency of utilization and processing in camellia oil in China. *China For. Sci. Technol.* **2001**, 6−9.

(18) Zhong, H. Y.; Wan, C. N.; Xie, B. X. Effect of supercritical CO_2 extracting conditions on the quality of oil-tea camellia seed oil. *J. Chin. Cereals Oils Assoc.* **2001**, 23−26.

(19) List, G. R. Decreasing trans and saturated fatty acid content in food oils. *Food Technol.* **2004**, *58*, 23−31.

(20) Gurr, M. I. Dietary fatty acids with trans unsaturation. *Nutr. Res. Rev.* **1996**, *9*, 259−279.

(21) Hulshof, K.; Van Erp-Baart, M. A.; Anttolainen, M.; Becker, W.; Church, S. M.; Couet, C.; Hermann-Kunz, E.; Kesteloot, H.; Leth, T.; Martins, I.; Moreiras, O.; Moschandreas, J.; Pizzoferrato, L.; Rimestad, A. H.; Thorgeirsdottir, H.; Van Amelsvoort, J. M. M.; Aro, A.; Kafatos, A. G.; Lanzmann-Petithory, D.; Van Poppel, G. Intake of fatty acids in Western Europe with emphasis on trans fatty acids: The transfair study. *Eur. J. Clin. Nutr.* **1999**, *53*, 143−157.

(22) Oomen, C. M.; Ocke, M. C.; Feskens, E. J. M.; Van Erp-Baart, M. A. J.; Kok, F. J.; Kromhout, D. Association between trans fatty acid intake and 10-year risk of coronary heart disease in the Zutphen elderly study: A prospective population-based study. *Lancet* **2001**, *357*, 746−751.

(23) European Food Safety Authority (EFSA). Opinion of the Scientific Panel on Dietetic, Products, Nutrition and Allergies on a request from the Commission related to the presence of trans fatty acids in foods and the effect on human health of the consumption of trans fatty acids. *EFSA J.* **2004**, *81*, 1−49.

(24) Baldioli, M.; Servili, M.; Perretti, G.; Montedoro, G. F. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1589−1593.

(25) Warner, K.; Neff, W. E. Chemistry of fried food flavours. In *Food Flavors and Chemistry, Advances of the New Millenium*; Spanier, A. M., Shahidi, F., Parliament, T. H., Mussinan, C., Ho, C.-T., Tratras Contis, E., Eds.; RSC: Cambridge, 2001.

(26) Zhong, H.; Bedgood, D.; Bishop, A.; Prenzler, P.; Robards, K. Endogenous biophenols, fatty acid and volatile profiles of selected oils. *Food Chem.* **2006**, in press.

(27) Gulcin, I. Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). *Toxicology* **2006**, *217*, 213−220.

(28) Chimi, H.; Cillard, J.; Cillard, P.; Rahmani, M. Cinetic of degradation for fatty-acids and phenolic-compounds in micellar solution. *Rev. Francaise Corps Gras* **1991**, *38*, 225−231.

(29) Chimi, H.; Cillard, J.; Cillard, P.; Rahmani, M. Peroxyl and hydroxyl radical scavenging activity of some natural phenolic antioxidants. *J. Am. Oil Chem. Soc.* **1991**, *68*, 307−312.

(30) Gomez-Alonso, S.; Fregapane, G.; Salvador, M. D.; Gordon, M. H. Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. *J. Agric. Food Chem.* **2003**, *51*, 667−672.

(31) Loots, D. T.; Van Der Westhuizen, F. H.; Jerling, J. Polyphenol composition and antioxidant activity of Kei-Apple (Dovyalis Caffra) juice. *J. Agric. Food Chem.* **2006**, *54*, 1271−1276.

(32) Marinova, E. M.; Yanishlieva, N. V.; Toneva, A. G. Antioxidant activity and mechanism of action of ferulic and caffeic acids in different lipid systems. *Riv. Ital. Sostanze Grasse* **2006**, *83*, 6−13.

(33) Nissiotis, M.; Tasioula-Margari, M. Changes in antioxidant concentration of virgin olive oil during thermal oxidation. *Food Chem.* **2002**, *77*, 371−376.

(34) Wang, M.; Jin, Y.; Ho, C. T. Evaluation of resveratrol derivatives as potential antioxidants and identification of a reaction product of resveratrol and 2,2,-diphenyl-1-picrylhydrazyl radical. *J. Agric. Food Chem.* **1999**, *47*, 3974−3977.

(35) Chen, C. W.; Ho, C. T. Antioxidant properties of polyphenols extracted from green and black tea. *J. Food Lipids* **1995**, *1*, 35−46.

(36) Pinelo, M.; Manzocco, L.; Nunez, M. J.; Nicoli, M. C. Interaction among phenols in food fortification: Negative synergism on antioxidant capacity. *J. Agric. Food Chem.* **2004**, *52*, 1177−1180.

(37) Morales, M. T.; Rios, J. J.; Aparicio, R. Changes in the volatile composition of virgin olive oil during oxidation: Flavors and off-flavors. *J. Agric. Food Chem.* **1997**, *45*, 2666−2673.

(38) McDonald, S.; Prenzler, P. D.; Antolovich, M.; Robards, K. Phenolic content and antioxidant activity of olive extracts. *Food Chem.* **2001**, *73*, 73−84.

(39) Pellegrini, N.; Visioli, F.; Buratti, S.; Brighenti, F. Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. *J. Agric. Food Chem.* **2001**, *49*, 2532−2538.

(40) Andrikopoulos, N. K.; Kalogeropoulos, N.; Falirea, A.; Barbagianni, M. N. Performance of virgin olive oil and vegetable shortening during domestic deep-frying and pan-frying of potatoes. *Int. J. Food. Sci. Technol.* **2002**, *37*, 177−190.

(41) Brenes, M.; Garcia, A.; Dobarganes, M. C.; Velasco, J.; Romero, C. Influence of thermal treatments simulating cooking processes on the polyphenol content in virgin olive oil. *J. Agric. Food Chem.* **2002**, *50*, 5962−5967.

Received for review July 15, 2006. Revised manuscript received October 6, 2006. Accepted October 8, 2006. Funding from National Natural Science Foundation of China (Grant No. 30571521) is gratefully acknowledged.

JF061974Z