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Endogenous biophenol, fatty acid and volatile profiles of selected oils

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Abstract

Fatty acid composition, total phenols, phenolic and volatile profile data are presented for cold-pressed and refined camellia oil, in comparison to other commercially available oils – avocado, pumpkin, sesame and soybean – representing a cross-section of bulk, ''bland'' oils (e.g., soybean) through to more boutique oils consumed primarily for taste (e.g., pumpkin). Camellia oil has a high oleic acid content, low polyunsaturated acid content, and levels of endogenous phenols comparable, in quantity and diversity, to cold-pressed oils. Volatile profiles of camellia oil are also comparable to cold-pressed oils, in that alkanals are the dominant headspace compounds, as measured by solid-phase microextraction gas chromatography. These factors suggest that camellia oil may find much wider commercial acceptance outside its current market range, southern China.

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1. Introduction

The food and agriculture organization of the United Nations (FAO) defines oil-bearing crops to include both annual (usually called oilseeds) and perennial plants whose seeds, fruits or mesocarp and nuts are valued mainly for the edible or industrial oils that are extracted from them. On this basis, the FAO lists 21 oil crops, with an annual world oilseed production of approximately 100 million tons [\(For](#page-6-0)[eign Agricultural Service USDA, 2005](#page-6-0)). Production of soybean dominates the industry, with cottonseed, peanut, sunflower and rapeseed/canola as other significant contributors. Only 5–6% of this production are used for seed (oilseeds) and animal feed, while about 8% for food. The remaining 86% is processed into oil, as salad and cooking oils, and for the production of margarine, shortening and compound fat.

A number of oils are produced in much lesser quantities but are nevertheless of significance regionally or because

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they impart particular flavours. For instance, seeds of camellia (Camellia oleifera Abel, Theaceae) have been utilized in China for more than 1000 years [\(Ruter, 2002\)](#page-6-0). Oil content of the seed is 40–50% and the extracted camellia seed or tea oil is the main cooking oil in China's southern provinces, especially Hunan, where more than 50% of the vegetable cooking oil is from camellias. Chinese production of camellia oil increased approximately 10 fold through the 1990s, from about 150,000 tonnes in 1990 [\(Ruter, 2002; Tang, Bayer, & Zhuang, 1993](#page-6-0)). Camellia oil is a high quality cooking oil, with a unique flavour and taste, good storage stability and claimed health benefits [\(Zhong, Wan, & Xie, 2001\)](#page-7-0). There is a long tradition of growing pumpkin (Cucurbita pepo L., Cucurbitaceae) as an oil crop in the Sajerska region in northeastern Slovenia and southern Austria ([Bavec, Gril, Grobelnik-Mlakar, &](#page-6-0) [Bavec, 2002\)](#page-6-0). The seed oil is used for salad dressings but also has uses in pharmacology and alternative medicine [\(Wagner, 2000](#page-7-0)). Similarly, avocado oil is recognised for its food uses [\(Moreno, Dorantes, Galindez, & Guzman,](#page-6-0) [2003\)](#page-6-0), but has also been identified for its potential in cosmetics and skin-care products [\(Athar & Nasir, 2005](#page-6-0)).

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Data on camellia, pumpkin and avocado oils are scarce in the refereed literature, in contrast with the vast literature on the high volume oils. For instance, the fatty acid profile of an oil ([Desouza, Matsushita, & Pereira, 1987;](#page-6-0) [Ghaleb, Farines, & Soulier, 1991\)](#page-6-0) has traditionally provided information on oil quality and has assumed increased importance in recent years [\(List, 2004](#page-6-0)). Interest in micronutrients, such as phenols, in oils has also increased. This can be attributed to the importance of phenols as antioxidants in the oil and their dietary effects when consumed by humans ([Koski et al., 2002](#page-6-0)). However, it is assumed that the polar phenols are mostly removed during processing of edible oils and their residual levels in refined oils are of considerable nutritional and epidemiological significance.

Of the three oils, avocado oil has the largest presence in the scientific literature but even here the data are more concerned with its health properties ([Swisher, 1988](#page-6-0)) and issues of adulteration [\(Werman, Mokady, & Neeman, 1996](#page-7-0)) than fundamental aspects of characterisation [\(Nieto & Romero,](#page-6-0) [1995; Werman & Neeman, 1987](#page-6-0)). Fatty acid data on camellia oils are surprisingly limited ([Xu, Meguro, & Kaw](#page-7-0)[achi, 1995a](#page-7-0)). There are some data on the phenolic profile of pumpkin seed oil ([Fruhwirth, Wenzl, El-Toukhy, Wagner,](#page-6-0) [& Hermetter, 2003](#page-6-0)) but no phenolic data on camellia or avocado oils.

This paper examines the fatty acid, phenolic and volatile profiles of six commercial camellia, pumpkin and avocado oils. These are compared with refined sesame oil and refined soybean oil, the latter being the dominant commercial oil.

2. Materials and methods

2.1. Materials

Refined pumpkin, avocado, soybean and camellia oil were provided by Soyatech International Ltd. (Queensland, Australia). Cold-pressed camellia oil was a commercial sample obtained from Hunan, P.R. China. Cold-pressed oils and pumpkin and avocado fruits were purchased from a local supermarket. All analyses were performed in at least duplicate.

2.2. Sample preparation

Oil samples were used without sample treatment other than dissolution in hexane. Fruit samples were extracted as previously described [\(McDonald, Prenzler, Antolovich,](#page-6-0) [& Robards, 2001\)](#page-6-0). The crushed flesh or seed $(10 g)$ was mixed in a beaker with methanol: water (25 mL; 70:30 v/ v for flesh, or 100:0 v/v for seed) and left to stand for 30 min. The extract was filtered and washed with hexane $(3 \times 25 \text{ ml}, \text{ or } 1 \times 25 \text{ ml})$ to remove oil. An aliquot of the extract was filtered through a $0.45 \mu m$ plastic non-sterile filter, prior to determination of total phenols and phenolic profile.

2.3. Determination of total phenols

Oil (3 g) was dissolved in hexane (15 ml) and extracted with methanol $(3 \times 5 \text{ ml})$ by shaking for 2 min for each extraction. The sample was left to stand overnight. The methanolic extract was washed with hexane (25 ml) and an aliquot (1 ml for oil extracts, 3 ml for flesh or seed) 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 against a reagent blank was measured using a Cary 50 spectrophotometer (Varian, Melbourne, Australia). Calibration was performed using caffeic acid and a calibration curve was obtained ($y = 124.3x + 0.080$; $R^2 = 0.9988$ in the range 0 to 100 μ g/10 ml).

2.4. Phenolic profiles

Oil (15 g) was dissolved in hexane (15 ml) and then extracted three times with methanol + water (1 ml; 50:50) v/v). The aqueous phase was then washed with hexane $(3 \times 3 \text{ ml})$. The hexane was discarded and the aqueous phase was filtered through a 0.45 µm plastic non-sterile filter, prior to high performance liquid chromatography (HPLC) analysis, which was performed using a Varian 9012 pump equipped with a 20 µ sample loop injector. The column eluent was monitored through a Varian 9065 Polychrom detector at 278, 259 and 240 nm. Separation was achieved on a Phenomenex (Lane Cove, Australia) C18 column (150 mm \times 4.6 mm; 5 µm) with gradient elution at a mobile phase flow rate of 1 mi 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 $40-50\%$ solvent B in 15 min, to 100% solvent B in 10 min, 5 min isocratic and returned to initial conditions over 10 min.

2.5. Volatile profiles

The volatile compounds from different oils were extracted using solid-phase micro extration (SPME) with a polydimethylsiloxane/Carbowax/divinylbenzene fiber (PDMS/CW/DVB, 50/30 lm, Supelco) as follows. Oil (1 g) was placed in a 10 ml reactivial (Supelco, Bellefonte, USA) and equilibrated at 40° C for 15 min. The SPME needle was inserted through the septum and left in the headspace at the same temperature for 30 min. The fibre was retracted into the needle and immediately transferred into the gas chromatograph. A Varian Star 3400 gas chromatograph with a BPX5 column $(30 \text{ m} \times 0.25 \text{ mm})$ i.d. \times 0.25 µm film; SGE, Ringwood, Australia) was used. The column was temperature programmed from an initial

temperature of 40 °C for 8 min, increased at $5 \text{ }^{\circ}\text{C/min}$ to $200 \degree C$ with a final isothermal period of 10 min. The fibre was desorbed for 1 min in a splitless injection port at 250 °C. The flow rate of nitrogen carrier gas was 2 ml min⁻¹. Chromatograms were routinely monitored by a flame ionization detector (FID) which was maintained at $300 \degree C$. For identification, volatiles were analyzed by gas chromatography–mass spectrometry (GC–MS) by thermal desorption in the injection port of a Varian 3400CX gas chromatograph (Varian) coupled with a Saturn 2000 ion trap mass spectrometer, using the same chromatographic conditions as before. 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 25,000 counts, and filament emission current was $15 \mu A$ with the axial modulation amplitude at 4.0 V. The ion trap temperature was maintained at $250 \degree C$ and the manifold temperature was maintained 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).

2.6. Fatty acid analyses

The fatty acid profile was determined as fatty acid methyl esters by gas chromatography. The methyl esters were prepared using the method prescribed by the IOOC [\(International Olive Oil Council, 2001](#page-6-0)), 0.2 ml of methanolic potassium hydroxide (0.2 M) was added to 0.1 g oil in 2 ml n-heptane. The mixture was shaken vigorously and allowed to stand and separate. An aliquot (1 ml) of the heptane phase was removed and dissolved in a further 1 ml n-heptane. Separation of fatty acid esters was performed on a Varian 3800 Gas Chromatograph with a Supelco SP 2340 capillary column (60 m \times 0.25 mm ID \times 0.2 µm film). The column temperature was programmed at 165° C for 10 min, 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. Flame ionization detector temperature was 245 °C. Data was analysed using star workstation chromatography software (Version 4.51). Quantification was performed by

external calibration. Relative errors were less than 1% at all concentration levels.

3. Results and discussion

Oils examined in this study were camellia, avocado, pumpkin, soybean and sesame oils. The oils were characterised by low peroxide values, ranging from 0.0 to 0.1 meq Kg^{-1} , iodine values of 83–133 Wijs and free fatty acid values of 0.02–1.5% (expressed as oleic acid).

3.1. Fatty acid profiles

Table 1 shows the fatty acid composition of the crude and refined oils. The oils exhibited wide variations in the contents of palmitic, palmitoleic, stearic, oleic and linoleic acids, leading to differences in total saturated and unsaturated fatty acids, monounsaturated and polyunsaturated fatty acids. Generally, the oils in this study had fatty acid profiles (Table 1) consistent with those published by the American Oil Chemists' Society (AOCS). All oils had high amounts of total unsaturated fatty acids (primarily oleic and linoleic acids) and are suitable for edible cooking or salad oils and for manufacture of margarine. Refining may have a small effect on the fatty acid profile [\(Moreno](#page-6-0) [et al., 2003; Ozdemir & Topuz, 2004\)](#page-6-0) but direct comparisons between profiles of the refined and cold-pressed/crude oils are not possible as different oil batches were purchased from the suppliers.

Fatty acid composition separated the oils into two groups, as those with high oleic acid contents (avocado and camellia oils) and those in which both oleic and linoleic acid contents were high were comparable (pumpkin, soybean and sesame oils). Avocado oil was unique in this group in containing significant amounts of palmitoleic acid, with levels comparable to those reported in other studies ([Moreno et al., 2003; Ozdemir & Topuz, 2004](#page-6-0)). Little has been reported in the refereed literature on the fatty acid profile of camellia oil the exception being two papers in Japanese ([Xu, Meguro, & Kawachi, 1995b\)](#page-7-0). In the non-refereed literature, e.g., company trade specifications, the oleic acid content of camellia oil is generally given as above 80%. Along with this high content of monounsatu-

rated lipid, camellia oil has the lowest levels of saturated fats.

The dominant fatty acids in pumpkin seed oil were linoleic acid, oleic acid, palmitic acid and stearic acid, similar to previously reported profiles [\(Younis, Ghirmay, & Al-](#page-7-0)[Shihry, 2000; Yu, Zhao, Chen, & Shu, 2004](#page-7-0)). The fatty acid profile of pumpkin seed oil was similar to that of the more common sesame oil. Soybean oil was distinguished by having the highest levels of linolenic acid. It is interesting to compare these profiles with large volume commercial oils, such as canola and olive oils (Table 2). By the 1980s polyunsaturated oils were being promoted as a healthy alternative to saturated products. At the same time, evidence was emerging of health problems associated with polyunsaturated fatty acids found in soybean oil and which also reduced oil stability and shelf life. Hydrogenation provided a solution to the problem of high polyunsaturates but produced undesirable trans-fatty acids ([Ayorinde, Garvin, &](#page-6-0) [Saeed, 2000; Sundram, Sambanthamurthi, & Tan, 2003;](#page-6-0) [Xu, Tran, Palmer, White, & Salisbury, 1999\)](#page-6-0). Studies had linked olive oil with improved coronary health and so the industry embraced monounsaturated fatty acids. The first colloquium on monounsaturates was held in Philadelphia and the second in Bethesda, Maryland in 1987. Olive oil was too expensive to produce for general use and world supplies of olive oil were insufficient to meet the demand for a healthy oil. Canola oil met the need with low polyunsaturates and high content of monounsarurates and omega-3 fatty acids. The profile for camellia oil makes it a direct competitor with canola oil and particularly olive oils (not incorporated in this study). Although the oleic and linoleic acid contents of avocado and canola oils are comparable, the higher saturated content of avocado oil is regarded as undesirable from a health perspective.

3.2. Phenol content

The total phenol contents of the oils are shown in Table 3. There was little difference in the total phenol content of the various oils. However, it is notable that the coldpressed and crude oils had slightly higher total phenol content than that of refined oils, with the exception of avocado oils.

Data for avocado, camellia, pumpkin and soybean oils are taken from [Table 1](#page-2-0) (rounded off) and other data from AOCS.

Data are reported as mean \pm standard deviation (CV%) and total peak area in HPLC chromatograms at 280 nm.

The transfer of phenols from seed and fruit to oil is of interest and data are also presented in Table 3 for phenol contents of selected fruits. When the seed of avocado was ground, its colour changed from white into red very quickly. No physical changes were observed during extraction of phenols from other samples. Methanol provided higher extraction efficiency from the fruits than aqueous methanol (data not shown). For instance, total phenol recovery (as caffeic acid) from avocado flesh was 504 ± 18 µg/g with methanol but only 378 ± 6 µg/g with 70% aqueous methanol. Total phenol content of the seeds was greater than that of the corresponding flesh. It appears that little of the phenol content is transferred to the oil.

Phenolic profiles were routinely collected by HPLC at 280 nm. Chromatograms for refined oils showed few, if any, definable peaks (chromatograms not shown) whereas the profiles of cold-pressed camellia, pumpkin, avocado and sesame oils were characteristic and distinctive ([Fig. 1](#page-4-0)). Peaks identified in the chromatograms were tyrosol (retention time 9.0 min), vanillic acid (12.0 min), caffeic acid (12.2 min) and o-coumaric acid (25.3 min). The relatively common phenolic compound, ferulic acid (18.7 min), was not detected in any of the oils. The complexity of the chromatograms which reflects phenol diversity changed in the sequence; pumpkin seed $>$ camellia $>$ sesame > avocado oil.

The chromatogram of the pumpkin seed oil displayed the hump typical of most reversed phase separations of phenols, but this was not observed in the other oils. This is likely to be derived from the seeds, as seen by comparing the chromatograms of seed, flesh and oil [\(Fig. 2](#page-4-0)). The origin of this hump is debatable but it is probably due to polymeric material.

From phenolic profiles, total phenols can be assessed as the summed area response in HPLC chromatograms. Data shown in Table 3 were from chromatograms obtained at 280 nm, which represents a compromise wavelength for detection of phenols. Trends were similar to those shown in the colorimetric data, although the observed effects of

Fig. 1. HPLC chromatograms comparing the phenolic profiles of cold-pressed avocado, camellia, pumpkin, and sesame oils at 280 nm. Other conditions as described under [Section 2.](#page-1-0) Major peak at 4.0 min is gallic acid added as an internal standard.

processing on total phenols were enhanced. For example, no peaks were detected for the refined camellia or avocado oils. Colorimetric data for total phenols were typically higher than corresponding data from HPLC due to interferences from vitamin C, for example, in the colorimetric method.

Fig. 2. HPLC chromatograms comparing the phenolic profiles of pumpkin cold-pressed oil, flesh and seed at 280 nm. Other conditions as described under [Section 2](#page-1-0).

Fig. 3. Volatile profiles of A cold-pressed/crude, and B refined oils using SPME and GC–MS. Conditions as described under [Section 2](#page-1-0). Compounds are identified as: 1. acetic acid (retention time in min 1.83; base peak in mass spectrum 43); 2. epoxide of short chain alkene (1.91; 41); 3. 3-methylbutanal (2.40; 43); 4. 2-butenal (2.44; 39) 5. 2-methylbutanal (2.52; 41); 6. 1-penten-3-ol (2.79; 57); 7. pentanal (2.95; 43); 8. 3-methyl-1-butanol (3.79; 55); 9. pentenal isomer (4.06; 55); 10. pentenal isomer (4.37; 55); 11. toluene (4.52; 91); 12. methylpentenal (4.82, 55) 13. 3-methyl-2-butanol (5.12; 45); 14. 2-pentanol (5.47; 45); 15. hexanal (5.80; 41); 16. furfural (7.52; 95); 17. (E)-2-hexenal (8.65; 39); 18. 1-hexanol (9.85; 56); 19. a nonene (10.7; 55); 20. heptanal (11.4; 55); 21. 2,5-dimethylpyrazine (12.0; 108); 22. ethylpyrazine (12.4; 107); 23. 1,3-nonadiene (12.5; 67); 24. a-pinene (12.7; 93); 25. benzaldehyde (13.8; 105); 26. 2,4 nonadiene (14.0; 67); 27. unknown alkene (14.0; 55); 28. (E)-2-heptenal (14.1; 41); 29. β -pinene (14.6; 93); 30. hexanoic acid (15.2; 60); 31. unknown alkene (15.3); 32. 1,3,5-trimethylbenzene (15.4; 105); 33. 2-pentylfuran (15.5; 81); 34. (E,E)-2,4-heptadienal (15.8; 81); 35. an octenol, possibly 2-octen-1-o1 (15.9; 81); 36. octanal (16.0; 41); 37. 2-ethyl-6-methylpyrazine (16.0; 121); 38. 3-carene (16.1; 93); 39. benzyl alcohol (17.2; 79); 40. a terpene (17.8; 93); 41. 3-ethyl-2,5-dimethylpyrazine (19.1; 135); 42. nonanal (19.9; 41); 43. 2-phenyl ethanol (20.1; 91).

Table 4

Total area counts and number of peaks in the volatile profiles of various oils obtained by SPME GC–FID

Sample	Total area counts $\times 10^5$	Number of peaks
Avocado oil refined	17	6
Avocado oil cold-pressed	7.2	16
Camellia oil refined	2.2	9
Camellia oil cold-pressed	10.2	27
Pumpkin oil refined	10.7	23
Pumpkin oil cold-pressed	19.5	24
Soybean oil refined	4.9	12
Sesame oil cold-pressed	3.5	10

3.3. Volatile profile

Headspace SPME provided a rapid and highly reproducible method for measurement of volatiles that avoided the use of large volumes of organic solvents. Milder temperatures used in SPME avoided the need to heat samples as in some recovery procedures and hence minimised the opportunity for artefact formation. Gas chromatograms for the various refined and crude oils are shown in Fig. 3, showing peak identities established from retention and mass spectral data. Among the 8 oil samples, total area counts in GC–FID (Table 4) covered a 10-fold range from 1.7×10^5 to 19.5×10^5 . In each case, refined oils had a lower total peak area than the corresponding crude or cold-pressed oil. Cold-pressed pumpkin oil and refined avocado oil had the highest and lowest total area counts, respectively. As for number of peaks in the chromatograms, the cold-pressed camellia oil ranked first. The order of the others was the same as that of total area counts (Table 4).

The most notable feature of the data is the presence of the series of straight chain alkanals in all oils in concentrations ranging from 1.2 to 16.1 mg/kg. Hexanal arises from linoleic acid, whereas octanal and nonanal are oleic acid derivatives. The difficulty of relating volatile content to

oil characteristics is demonstrated by Moreno et al. (2003), in which the volatile profile of avocado oil was closely dependent on the extraction procedure. Nevertheless, hexanal levels were generally lower in avocado and camellia oils, in which there was a higher oleic acid content. Hexanal is also characteristic of oxidation processes and many oxidation products have been reported (Sinyinda & Gramshaw, 1998) in the fresh mesocarp of avocado. Strecker aldehydes such as 2- and 3-methylbutanal were restricted to cold-pressed camellia and cold-pressed pumpkin oils. Pyrazines were observed only in cold-pressed pumpkin oil and have been previously identified as character impact compounds in this oil (Matsui, Guth, & Grosch, 1998). Furans such as 2-pentylfuran observed in refined pumpkin oil generally impart caramel-like and roasted odours (Shimoda, Nakada, Nakashima, & Osajima, 1997) and the absence of these pyrazines and furans in sesame seed oil suggests that the oil used in the present study was produced with minimal roasting of sesame seeds.

The numbers of peaks were not changed significantly between cold-pressed and refined pumpkin oils, but the major peaks differed between the two oils, producing significantly different volatile, profiles, as seen in [Fig. 3](#page-5-0). There were significantly fewer volatiles in both oils than reported for roasted pumpkin seeds by Siegmund and Murkovic (2004). This is not surprising given the importance of roasting temperature to the production of volatiles and particularly those derived from pyrazine. Matsui et al. (1998) reported 24 odour-active compounds in pumpkin seed oils. In contrast, profiles of cold-pressed and refined camellia oils and cold-pressed and refined avocado oils were qualitatively similar. In the case of the avocado oils, the effect of processing was to cause a general reduction in the amount of all volatiles, whereas selective changes were observed for the camellia oils. Thus, pentanal was the major volatile in refined camellia oil, whilst hexanal was the major volatile in cold-pressed camellia oil.

The data presented here confirm the low levels of phenols in the refined oils and provide a basis for further investigations of these oils. In comparison with the other oils, camellia oil has a 'good' fatty acid profile and its phenols content and diversity are comparable to that of pumpkin seed oil. The results indicate that cold-pressed camellia oil represents a viable option, since the high oleic acid content combined with low polyunsaturated content and good phenol content mean that rancidity during processing will be minimised and phenols will provide stability on storage.

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