Original paper

Characterization of Aroma Volatiles in Camellia Seed Oils (*Camellia oleifera* Abel.) by HS-SPME/GC/MS and Electronic Nose Combined with Multivariate Analysis

Wenming CAO¹, Lang LIN¹, Yunwei NIU^{1*}, Zuobing XIAO¹ and Xuezhi FANG²

¹School of Perfume and Aroma Technology, Shanghai Institute of Technology, Shanghai 201418, PR China

²Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Fuyang 311400, PR China

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Volatile compounds of Camellia seed oils from 5 different varieties were characterized by headspace solid phase microextraction/ gas chromatography/mass spectrometry (HS-SPME/GC/MS). Four parameters of SPME were optimized and the best extraction conditions were: 50/30 μ m DVB/CAR/PDMS fiber, 45°C and 45 min of SPME, and 4.00 g sample size. Forty-six volatiles were identified, including 16 aldehydes, 10 alcohols, 4 acids, 7 ketones, 3 terpenes and 6 esters. Further correlation analysis between aroma compounds and sensory attributes revealed pentanal, hexanal, (*E*)-2-hexenal, octanal, (*E*)-2-heptenal, nonanal, (*E*)-2-octenal, (*E*)-2-decenal, (*E,E*)-2,4-heptadienal, 2-hexanol, 2-heptanol, hexanol, octanol, acetic acid, pentanoic acid, 1-*p*-menthene, limonene, γ -terpinene, ethyl-2-methylbutanoate, 2-methylbutyl acetate, ethyl 2-methyl-2-butenolate, γ -butyrolactone, γ -hexalactone were the important characteristic aroma compounds of Camellia seed oils. Additionally, electronic nose (e-nose) was applied to analyze the aroma of Camellia seed oils. HS-SPME/GC/MS and e-nose techniques combined with multivariate analysis can be used to distinguish between different Camellia seed oils.

Keywords: camellia seed oil, aroma compounds, HS-SPME/GC/MS, electronic nose

Introduction

The genus *Camellia* belongs to the Theaceae, a family of woody shrub. They are economically important for human consumption. In western countries, *Camellia* species cultivated as ornamentals (e.g., *C. japonica*, *C. reticulata*, and *C. sasanqua*) (Vela *et al.*, 2013). *Camellia oleifera* Abel. has the largest plantation area and the highest annual oil yield of all of the woody oil plants in China. The seed of *Camellia oleifera* Abel. can be pressed to obtain high quality oils that are used extensively for cooking (Su *et al.*, 2014). Camellia seed oil is similar to olive oil in chemical composition (Ali Sahari *et al.*, 2004). It contains abundant unsaturated fatty acids consisting of oleic acid and linoleic acid, vitamin E, squalene, flavonoids (He *et al.*, 2010). The oleic acid content in the oil can reach as much as 80%. The high oleic content of Camellia seed oil has health-promoting effects including

lowering blood pressure, cholesterol and triglycerides (He *et al.*, 2010; Zeb, 2012). Additionally, Camellia seed oil contains some natural antioxidants with biological activities (Lee & Yen, 2006).

Volatile compounds play an important role for the aroma quality of oils. Gas chromatography-mass spectrometry (GC/MS) can easily identify the compounds of a mixture and offers high resolution for organic volatile compounds detection and high reproducibility. Reboredo-Rodríguez *et al.* (2012) identified and quantified simultaneously fifty-one characteristic volatile compounds from extra-virgin olive oils by GC/MS. De los Angeles Fernandez *et al.* (2014) also used HS-SPME/GC/MS associated with multivariate analysis to characterize the compound volatile profile of the most representative olive oil cultivars. Through HS-SPME/GC/MS analysis, Jelen *et al.* (2000) characterized the volatile compounds in vegetable oils of different sensory quality.

*To whom correspondence should be addressed.

E-mail: flavoracademysit@yeah.net

Electronic nose (e-nose) technology provides a nondestructive, easy and fast analysis method for odor analysis, which has been successfully applied to various food areas. Many studies have investigated the application of electronic nose for the aroma fingerprint of vegetable oils (Gan *et al.*, 2005; Guadarrama *et al.*, 2000; Hai & Wang, 2006; Taurino *et al.*, 2002). These studies demonstrated that e-nose was successfully applied to classify edible oils according to the cultivar, harvest year, geographical origin, and detect the adulteration.

Camellia seed oil has a delicate and unique flavor among various types of vegetable oils, and is highly appreciated by consumers. However, no report about e-nose about characterization of aroma volatiles in Camellia seed oil using HS-SPME/GC/MS and electronic nose combined with multivariate analysis has been available. The aim of the present work was to (a) characterize the volatile profile of five Camellia seed oil varieties using optimized HS-SPME-GC/MS; (b) investigate the relationship among varieties, aroma compounds and sensory attributes using partial least squares regression (PLSR); (c) make a comparison among the five Camellia seed oil varieties by electronic nose fingerprint analysis system combined with principal component analysis (PCA). A better comprehension of this knowledge will be significantly useful for distinguishing the characteristic aroma of Camellia seed oils from one another and identifying the genuine and fake Camellia seed oil.

Materials and Methods

Materials Camellia seeds (*Camellia oleifera* Abel.) of five different varieties were obtained from National Center for Oil-tea Camellia Science and Technology in Zhejiang province, China. These varieties were Changlin 21(CO21), Changlin 27 (CO27), Changlin 18 (CO18), Changlin 55 (CO55), Changlin 3 (CO3). Averaging 1.5 – 2.0 kg Camellia seeds of each variety were collected at the same time from trees grown under the same conditions. They were pressed by the hydraulic press to produce oil. Temperature of extracted oil was continuously at below 40°C.

Four different coating fibers for headspace solid-phase microextraction (HS-SPME), including 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 65 µm polydimethylsiloxane /divinylbenzene (PDMS/DVB), 100 µm polydimethylsiloxane (PDMS) and 75 µm carboxen/polydimethylsiloxane (CAR/PDMS), were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). 1, 2-Dichlorobenzene (internal standard), *n*-alkane standards (C7-C30) and other authentic reference compounds were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO).

Sensory evaluation Sensory analysis of five Camellia seed oils was conducted by a group of 9 well-trained panelists. The sensory panel was selected and trained according to the International Olive Oil Council regulation (IOOC, COI/T.20/Doc. No 15/Rev. 4, 2011). Descriptive analysis was used for odor profile of samples,

employing a 10-point interval scale (0 = none, 9 = extremely strong). Three specific training sessions were carried out. In the first session, panelists generated descriptive terms for the Camellia seed oil; in the second, different aroma standards were presented and discussed by panelists. From the discussion, five aroma terms, including green, sweet, fruity, woody, pungent were selected for further sensory evaluation. In the third session, each panelist evaluated all the oil samples on a 10-point interval scale mentioned above. The sensory sessions performed in a sensory laboratory, which conformed to international standards for test room.

Optimization of SPME conditions An SPME fiber was used for the extraction after the fiber had been conditioned. Four grams of oil was placed into a 15 mL headspace vials and 2 µL of 1, 2-dichlorobenzene (60.0 µg /mL in absolute methanol) was spiked directly into the oil vials as internal standard to facilitate quantitative analysis. Then the SPME fiber was inserted through the septum and exposed to the headspace. The fiber was immediately transferred into the gas chromatograph injector for desorption for 5 min at 250°C in the splitless mode.

In order to improve volatile compounds absorption, the following experimental parameters were investigated: four different coating fibers, extraction temperature, extraction time, and oil sample size. The analysis was conducted in triplicate for each parameter investigation.

GC/MS analysis A gas chromatograph (Hewlett Packard 7890) and HP 5973C MS detector (Agilent Technologies, USA) were used to analyze volatile aroma components. Chromatographic separations were performed on an HP-INNOWAX fused silica capillary column (60 m × 0.25 mm ID, 0.25 µm film thickness). High purity helium as carrier gas was circulated at 1 mL min⁻¹ in the constant flow mode. The injection was conducted in a splitless mode for 5 min at 250°C. The oven temperature program was as follows: 40°C for 3 min; 3°C/min ramp to 180°C and holding for 5 min; 8°C/min ramp to 200°C and holding for 10 min. The transfer line temperature was 250°C, and the ion-trap manifold temperature 230°C. The quadrupole mass filter was set at 150°C. The ionization was performed in electron ionization (EI) mode at 70 eV. The chromatograms were recorded by monitoring the total ion currents in a mass/charge range of 30 – 450 amu.

The identification of the volatile compounds was achieved by comparing their retention index (RI) and mass fragmented patterns with those of standard compounds, or with mass spectrums in the Wiley7n, 1 Database (Hewlett-Packard, Palo Alto, CA) and NIST Database and previously reported retention indices in the literatures. The RI of volatile compounds was calculated by sample injection with a homologous series of straight-chain *n*-alkanes (C7-C30) (concentration of 1000 mg L⁻¹ in *n*-hexane) under the same conditions. The integrated areas based on the total ion current chromatogram were normalized to the areas of the internal standard and averaged. Each volatile concentration in the Camellia seed oil was determined by comparison with the concentration of the

internal standard. To quantify the volatiles, each sample was analyzed in triplicate and the average of the results was used for data analysis.

Electronic nose analysis Oils were analyzed by a FOX 4000 nose sensor from Alpha M.O.S. (Toulouse). The e-nose system comprised a sampling system, a detector system and processing system (Alpha MOS proprietary software), which utilized multivariate statistical analysis and chemometrics to acquire, compute and interpret electronic nose mensuration. The sensory array was composed of 18 metal oxide gas sensors consisting of three types of sensors: LY-type, T-type and P-type. LY sensors are chromium–titanium oxides ($\text{Cr}_{2-x}\text{Ti}_x\text{O}_{3-y}$) and tungsten oxide (WO_3) sensors. Types T and P are both based on tin dioxide (SnO_2) but have different sensor geometries. Multiple types of sensor are used in the instrument in order to ensure adequate sensitivity and selectivity. The condition is as following: 0.50 g Camellia seed oil sample was placed in 10 mL headspace vial and capped with a Teflon faced silicon rubber cap. After 30 min equilibration at room temperature, the vials were loaded into auto-sampler tray, and equilibrated for 15 min at 45°C under agitation (500 rpm). The Camellia seed oil sample headspace was pumped over the sensor surfaces for 120 s, which was sufficient for sensors to reach the stable values. After the analysis, the system was purged with pure air prior to the next oil sample injection, allowing the instrument to reestablish the base line. Each of the oil samples has been replicated four times.

Statistical analysis Data from the descriptive analysis was evaluated by analysis of variance (ANOVA) using IBM SPSS statistics software (v. 19.0, SPSS, Inc., Chicago, IL). ANOVA with Duncan's multiple comparison test was performed to determine whether there was difference among individual oil sample for each sensory attribute. The differences were considered to be significant at $p \leq 0.05$.

PLSR was applied to explore the relationship among oil varieties, sensory attributes and volatile compounds of five Camellia seed oils through Unscrambler ver. 9.7 (CAMO ASA, Oslo, Norway). All variables were centered and standardized so as to make each variable have a unit variance and zero mean before applying PLS analysis in order to obtain an unbiased contribution of each variable to the criterion. By applying PLSR analysis to standardized data, importance of peaks for each attribute could be compared quantitatively based on regression coefficients and loading weights for each predictor or X variable used in PLSR models. PCA was carried out to categorize the Camellia seed oils into different groups based on their aroma fingerprints obtained by e-nose. XLSTAT ver. 2010 (Addinsoft, New York, NY, USA) software was used for the analysis.

Results and Discussion

Optimization of SPME conditions The aroma compounds in Camellia seed oils were extracted using HS-SPME. Different

extraction conditions were investigated, including four different coating fibers, extraction temperature and time, and oil sample size. The optimization of experimental condition was based on the total ion current chromatogram in the GC/MS.

It showed four different fibers (50/30 μm DVB/CAR/PDMS, 65 μm PDMS/DVB, 100 μm PDMS and 75 μm CAR/PDMS) on the adsorption of volatile compounds from the Camellia seed oil (Fig. 1A). The results showed that there were significant differences among the four coating fibers and the DVB/CAR/PDMS fiber presented the largest peak area for the volatiles. Therefore, the DVB/CAR/PDMS fiber was selected for the experiments.

The effects of extraction temperature (35°C, 40°C, 45°C, 50°C) on the detection of total volatiles showed in Fig. 1B. The total peak area of volatiles significantly rose when the extraction temperature increased from 35°C to 45°C. However, there was a decrease at 50°C. This may be due to higher temperature leading to desorption from the fiber and mutual chemical reactions (Plutowska *et al.*, 2011). Thus, the extraction temperature was determined at 45°C for the best extraction efficiency.

The effects of extraction time on adsorptivity over the range of 15–60 min were shown in Fig. 1C. Maximum adsorptivity was observed at 45 min. Increasing sample extraction time above 45 min resulted in a significant decrease in peak area of volatiles. Desorption of analytes from the fiber with increasing extraction time during HS-SPME analysis may be attributed to reverse diffusion of analytes in order to maintain the partition equilibrium (Achouri *et al.*, 2006).

As can be seen in Fig. 1D, a set of experiments using 2.00, 4.00 and 6.00 g were carried out. An increase from 2.00 to 4.00 g in the extraction efficiency was observed. As the amount of sample increases, the headspace volume is minimized (Gorecki & Pawliszyn, 1997). There was a decrease in the extraction efficiency by using 6.00 g oil. A previous study has reported that reverse diffusion of analytes from fiber to sample could occur as a result of overloading of the fiber (Achouri *et al.*, 2006). Good extraction efficiency was achieved at 4.00 g oil for most volatiles.

On the basis of the above results, the following conditions were considered as the most suitable for the analysis of volatiles in Camellia seed oil: fiber, 50/30 μm DVB/CAR/PDMS; oil sample size, 4.00 g; extraction temperature, 45°C; and extraction time, 45 min.

SPME/GC/MS analysis of volatile compounds Volatiles of five Camellia seed oils were adsorbed by HS-SPME using the optimized conditions and analyzed by GC/MS. A total of 46 compounds belonging to different chemical classes were tentatively identified and then quantified, including 16 aldehydes, 10 alcohols, 4 acids, 7 ketones, 3 terpenes and 6 esters, as listed in Table 1. It is worth noting that different Camellia seed oils show differences in the volatile profiles. For instance, a total of 35 volatile compounds were identified and quantified in CO18, while 17 in CO27. The

Fig. 1. Effects of coating fibers (A), extraction temperature (B), extraction time (C), oil sample size (D) on the total peak areas for aroma volatiles in Camellia seed oil by SPME-GC/MS.

total concentration of these volatiles in five Camellia seed oils ranged from 765 $\mu\text{g/kg}$ to 6124 $\mu\text{g/kg}$. Thirteen volatiles among the total volatile compounds existed in all varieties.

In the present study, aldehydes were the most abundant volatile compounds and accounted for the largest proportion of the total concentration (except CO21), representing from 47.5% to 65.3%. Of the volatiles produced by the breakdown of the alkoxy radicals, aldehydes are the most significant flavor compounds in Camellia seed oil, coincidentally in olive oil and corn oil (Goicoechea & Guillén, 2014; Kesen *et al.*, 2013). Especially, it was reported that C6 aldehydes and alcohols are the most abundant compounds and contribute significantly to the flavor of virgin olive oil (Cavalli *et al.*, 2004).

In this work, hexanal, (*E*)-2-pentenal, heptanal, octanal and (*E*)-2-heptenal existed in all varieties were considered to enhance flavor quality, most of which were associated with green, sweet, fruity, nutty, oily and fatty odor (Krist *et al.*, 2006). Hexanal was identified as the main volatile compounds in five Camellia seed oils. CO18 accounted for the higher concentration of hexanal

(1676 $\mu\text{g/kg}$), followed by the CO3 (957 $\mu\text{g/kg}$), CO55 (905 $\mu\text{g/kg}$), CO21 (317 $\mu\text{g/kg}$), CO27 (223 $\mu\text{g/kg}$). Aparicio *et al.* (1997) reported that hexanal, (*E*)-2-hexenal were the major aroma compounds identified in most virgin olive oils from European countries. Similar results were also found by Kiritsakis (1998). (*E*)-2-pentenal showed the highest concentration in CO55 (107 $\mu\text{g/kg}$) and CO18 (104 $\mu\text{g/kg}$). (*E*)-2-hexenal was detected with concentration between 23.2 $\mu\text{g/kg}$ and 64.0 $\mu\text{g/kg}$ in oil samples. (*E*)-2-heptenal was identified in all Camellia seed oil samples at relatively high concentration. It was found that (*E*)-2-pentenal, (*E*)-2-hexenal, (*E*)-2-heptenal was related with the green aroma of linseed oil (Krist *et al.*, 2006). Straight chain saturated aldehydes such as heptanal, octanal and nonanal were identified with the highest concentration of 188 $\mu\text{g/kg}$, 252 $\mu\text{g/kg}$ and 368 $\mu\text{g/kg}$ in CO18, respectively. Pentanal, octanal and nonanal are responsible for the fresh and slightly green notes of the pumpkin seed and oil (Siegmund & Murkovic, 2004). Kesen *et al.* (2013) found that octanal with citrusy and lemon aroma was one of the most powerful aroma active compounds in olive oil from Memecik. Its

Table 1. Volatile compounds of 5 Camellia seed oil varieties identified by GC/MS.

code	compounds	RI ^a	ID ^b	RI ^c	concentration ^d (µg/kg)				
					CO21	CO55	CO27	CO3	CO18
	aldehydes								
A1	pentanal	974	A, B	963	50.7	149	30.9	246	NDe
A2	2-butenal	1039	B	1041	16.0	75.8	ND	ND	72.0
A3	hexanal	1078	A, B	1073	317	905	223	957	1676
A4	(<i>E</i>)-2-pentenal	1127	A, B	1121	33.3	107	10.3	58.5	104
A5	heptanal	1181	A, B	1180	41.3	124	34.3	169	188
A6	2-butenal, 3-methyl-	1195	B	1182	5.33	14.7	ND	ND	ND
A7	(<i>E</i>)-2-hexenal	1214	A, B	1213	29.3	23.2	ND	55.4	64.0
A8	octanal	1284	A, B	1280	22.7	86.3	12.6	151	252
A9	(<i>E</i>)-2-heptenal	1228	B	1243	108	141	20.6	234	532
A10	nonanal	1388	A, B	1385	ND	88.4	32.0	166	368
A11	(<i>E</i>)-2-octenal	1426	B	1436	53.3	27.4	ND	40.0	160
A12	(<i>E,E</i>)-2,4-heptadienal	1491	A, B	1496	21.3	202	ND	80.0	180
A13	benzaldehyde	1525	A, B	1527	44.0	56.8	ND	36.9	48.0
A14	(<i>E</i>)-2-Nonenal	1532	A, B	1536	33.3	18.9	ND	21.5	ND
A15	(<i>E</i>)-2-decenal	1631	B	1616	ND	ND	ND	ND	188
A16	(<i>E,E</i>)-2,4-Decadienal	1762	B	1763	12.0	19.0	ND	9.23	164
	alcohols								
B1	2-methyl-1-propanol	1086	B	1084	34.7	118	29.7	142	76.0
B2	butanol	1138	A	1145	17.3	42.1	ND	43.1	ND
B3	2-methyl-1-butanol	1198	A, B	1208	217	16.8	ND	30.8	180
B4	pentanol	1244	A, B	1255	268	152	ND	338	168
B5	2-hexanol	1287	B	1307	ND	ND	ND	ND	24.0
B6	2-heptanol	1310	B	1303	ND	ND	ND	ND	92.0
B7	hexanol	1346	A, B	1350	33.3	16.8	ND	27.7	112
B8	1-octen-3-ol	1443	B	1444	196	10.5	ND	24.6	240
B9	heptanol	1447	A, B	1457	109	29.5	ND	80.0	136
B10	octanol	1549	A, B	1553	ND	16.8	ND	27.7	ND
	carboxylic acids								
C1	acetic acid	1451	A,B	1450	135	543	93.7	388	196
C2	propanoic acid	1538	A	1523	34.7	505	36.6	98.5	104
C3	pentanoic acid	1718	A, B	1745	ND	27.4	6.86	27.7	ND
C4	hexanoic acid	1841	A, B	1840	12.0	84.2	19.4	129	56.0
	ketones								
D1	2-pentanone	973	A	983	32.0	50.5	16.0	58.5	60.0
D2	1-penten-3-one	1018	A	1016	ND	ND	ND	54.7	44.0
D3	2-heptanone	1178	A	1170	141	44.2	59.4	61.5	292
D4	2-octanone	1280	A, B	1285	37.3	23.2	77.7	43.1	152
D5	6-methyl-5-hepten-2-one	1334	B	1332	8.00	63.2	ND	9.23	12.0
D6	2-nonanone	1384	A	1388	34.7	14.7	42.3	27.7	44.0
D7	3-octen-2-one	1402	A	1388	30.7	ND	ND	ND	16.0
	terpenes								
E1	1- <i>p</i> -menthene	1123	A		ND	ND	ND	ND	8.00
E2	limonene	1190	A, B	1186	88.0	ND	19.4	ND	ND
E3	γ-terpinene	1240	A	1238	12.0	ND	ND	ND	ND
	esters								
F1	ethyl-2-methylbutanoate	1048	A	1042	ND	ND	ND	ND	20.0
F2	2-methylbutyl acetate	1116	A	1116	65.3	ND	ND	ND	ND
F3	ethyl 2-methyl-2-butenate	1160	A		257	ND	ND	ND	16.0
F4	octyl formate	1549	A		45.3	ND	ND	ND	80.0
F5	γ-butyrolactone	1634	A, B	1637	8.42	ND	ND	ND	ND
F6	γ-hexalactone	1704	A, B	1705	10.5	ND	ND	ND	ND

a Linear retention indices calculated of unknown compounds on a HP-INNOWAX capillary column (60 × 0.25 mm × 0.25 µm) with a homologous series of *n*-alkanes (C7-C30).

b Identification method: A=mass spectrum and RI agree with that of the authentic compounds run under similar GC/MS conditions; B=mass spectrum and RI agree with literature data.

c From the flavornet database (<http://www.flavornet.org>, accessed June 2007), Acree, 2004 (on C20M stationary phase); in the literature.

d Results are the average of triplicate as µg/kg. Standard deviation of all volatile compounds was below 10%.

e ND: not determined.

flavor dilution factor could reach up to 1024 at the concentration of 293 µg/kg. (*E*)-2-hexenal, (*E*)-2-octenal, (*E,E*)-2,4-heptadienal, benzaldehyde and (*E,E*)-2,4-decadienal were all absent in CO3. (*E*)-2-octenal, (*E,E*)-2,4-heptadienal and (*E,E*)-2,4-decadienal yielded green plant, fatty and solvent odors. Benzaldehyde, supposed to be Strecker degradation product of aromatic amino acids, mainly associated with aromatic, popcorn odor was found in the four Camellia seed oils with relatively lower amount. (*E*)-2-nonenal contributed to fruity, grassy aroma, was detected in CO21, CO55 and CO3. (*E*)-2-decenal gave a fatty aroma was only detected in CO18 at the concentration of 188 µg/kg. Most of these compounds could be produced during oil extraction via decomposition of hydroperoxide formed by the oleic, linoleic and linolenic fatty acids (Angerosa *et al.*, 2004; Dierkes *et al.*, 2012).

Alcohols are produced by the action of alcohol dehydrogenase (ADH) enzyme which is responsible for the formation of volatile alcohols. As shown in Table 1, 10 kinds of alcohols, B1 to B10, were detected. CO18 (1028 µg/kg) showed the highest concentration of total alcohols, probably related to higher activity of the ADH enzyme, followed by CO21 (876 µg/kg), CO3 (714 µg/kg) and CO55 (402 µg/kg). CO27 only had one alcohol (2-methyl-1-butanol) and little trace (29.7 µg/kg). Within the alcohols, 2-methyl-1-propanol was present in all oil samples. 1-octen-3-ol and pentanol were also found to be the main alcohols in corn oil stored at room temperature (Goicoechea *et al.*, 2014). 2-Hexanol and 2-heptanol were only identified in CO18 with low concentration. Hexanol, a common and important flavor component of some vegetables, has herbaceous, woody, green odor and was identified in CO21 (33.3 µg/kg) and CO18 (112 µg/kg).

It has been demonstrated that the organic acid is mainly formed through a hydrolytic β -dicarbonyl cleavage pathway by Davidek *et al.*, 2006). The total amount of carboxylic acids in CO55 and CO3 were significantly higher than that in other varieties. Among them, acetic acid was present at high concentrations (from 93.7 to 543 µg/kg) and its aroma contribution can be confirmed in linseed oil and camelina oil (Krist *et al.*, 2006). Acetic acid has been previously discussed in a variety of studies analyzing volatile compounds of different oil types (Jelen *et al.*, 2000; van Ruth *et al.*, 2000). Propanoic acid with pungent note has also been detected in the highest concentration (505 µg/kg) in CO55. The amount of pentanoic acid was low and ranged from 6.86 to 27.7 µg/kg. Hexanoic acid, mainly enzymatically produced from polyunsaturated fatty acids through the lipoxygenase pathway (Angerosa *et al.*, 2004), was identified with the concentration between 12.0 µg/kg and 129 µg/kg in all oil varieties.

Ketones might be formed by β -oxidation of fatty acids which generated some important flavor compounds (Yu *et al.*, 2008). Seven ketones were identified in Camellia seed oil samples (Table 1). Among them, 2-pentanone, 2-heptanone, 2-octanone, and 2-nonanone were all characterized in five oil varieties. Cavalla *et al.* (2004) found that C5 ketones are linked to positive sensory

properties. 2-Heptanone with sweet, fruity and cinnamon aroma was presented with the concentration varied from 9.33 µg/kg to 292 µg/kg in oils. 2-Nonanone was also detected in beech oil exhibited nutty, fatty and roasted flavor (Bail *et al.*, 2009).

Three terpenes, 1-*p*-menthene, limonene and γ -terpinene, were detected. The contents of them were relatively lower compared with aldehydes, alcohols, carboxylic acids, ketones. Among terpenes, γ -terpinene and 1-*p*-menthene were only identified in CO18 and CO21, respectively. Limonene with mild, citrus, sweet, orange, and lemon aroma was found in various oils. It may come from the rearrangement of sterols or squalene or their oxidation products (Kao *et al.*, 1998), and could play a very important role in the fragrance of the oil (Bail *et al.*, 2009; Zunin *et al.*, 2005). In this work, limonene was detected in CO21 (88.0 µg/kg) and CO27 (19.4 µg/kg).

Esters, derived from the esterification of free fatty acids and alcohols, have characteristic odor of sweet, fruity, herbaceous and honey. Six esters were detected in this work. Ethyl-2-methylbutanoate and 2-methylbutyl acetate were detected in CO21 and CO18, respectively. While octyl formate and 2-methyl-2-butenic acid, ethyl ester were all present in CO21 and CO18. γ -butyrolactone and γ -hexalactone were only presented in CO27 with low concentration. Interestingly, γ -butyrolactone and γ -hexalactone were previously found in linseed oil, camelina seed oil (Angerosa *et al.*, 2004; Krist *et al.*, 2006).

Relationship between Camellia seed oil samples, sensory attributes and aroma compounds The results of the sensory evaluation of 5 Camellia seed oil varieties are shown in Table 2. ANOVA analysis revealed that sweet, fruity, green, woody and pungent attributes had statistically significant differences ($p < 0.05$) designated with different letters. Duncan's multiple comparison test results revealed that pungent had most significant difference, with different superscripts for each sample. CO21 had the strongest fruity and sweet attributes, but it had the least weak pungent attribute. CO55 and CO27 had almost the same score in sweet, green and woody aroma, but the score of pungent note was higher in CO55. CO3 presented higher woody and pungent aroma. CO18 had the highest scores in green and woody aroma.

Mean data accumulated from sensory evaluation and GC/MS analysis was processed by ANOVA-PLSR. 46 volatile compounds were used as variables in the subsequent PLSR analysis. The X-matrix was designated as GC/MS profiles. The Y-matrix was designated as oil varieties and sensory attributes. The resultant correlation loadings plot of the first two components was shown in Fig. 2. For X variables, the explained variance for this model was PC1=40% and PC2=33%; for Y variables, the explained variance for this model was PC1 and PC2 with 40%, 27%, respectively. The big circles indicated 50% and 100% explained variance, respectively. All the volatile compounds, samples and five sensory attributes, except 2-butenal, 3-methyl-2-butenal, benzaldehyde, (*E*)-2-nonenal, pentanol, propanoic acid, 1-penten-3-one, 6-methyl-

Fig. 2. An overview of the variation found in the mean data from partial least squares regression (PLSR). Correlation loadings of indicator variables 46 volatile compounds (codes correspond to the compounds described in Table 1).

Table 2. The mean scores of the five attributes for 5 Camellia oils in sensory analysis.

Sensory attribute	CO21	CO55	CO27	CO3	CO18	Description
sweet	5.04 ± 0.85a	2.93 ± 1.00c	3.52 ± 1.05b	2.70 ± 0.87c	2.11 ± 0.75d	Aroma of baking chestnuts, peanuts and almonds
fruity	3.04 ± 0.81a	0.89 ± 0.75c	2.04 ± 0.98b	2.07 ± 1.11b	1.11 ± 0.97c	Smelling of fresh cut apple
green	3.33 ± 0.96c	4.44 ± 1.34b	3.00 ± 1.11c	3.26 ± 1.32c	6.15 ± 1.17a	The aroma characteristics of fresh grass
woody	4.70 ± 1.07bc	4.22 ± 1.05c	3.63 ± 1.01d	4.93 ± 0.96b	5.74 ± 1.02a	The scent of newly chopped woods
pungent	0.67 ± 0.83e	3.93 ± 1.03b	1.85 ± 1.13c	4.81 ± 1.24a	1.26 ± 0.94d	The aroma characteristics of chili pepper

Mean scores in the same row followed by different letters are significantly different ($p < 0.05$) ($n = 27$: 9 panelists with 3 replications).

5-hepten-2-one, CO55, CO3 and CO27, were located between the inner and outer ellipses, $r^2=0.5$ and 1.0, respectively. All the sensory variables were outside of the $r^2=0.5$ ellipse, indicating that the sensory attributes were well explained by the PLSR model.

As shown in Fig. 2, the contrast between fruity, sweet aroma on the negative dimension and pungent, green, woody aroma on the positive dimension are present in the x axis. Fruity, sweet attributes and CO21, located in the lower left hand quadrant, were correlated with one another. Moreover, CO21 was highly related to limonene (E2), γ -terpinene (E3), ethyl-2-methylbutanoate (F1), 2-methylbutyl acetate (F2), ethyl 2-methyl-2-butenate (F3), γ -butyrolactone (F5), γ -hexalactone (F6). In the opposite direction, CO18 was highly associated with green and woody aroma. It had good correspondence to the sensory evaluation result that CO18 had the strongest aroma intensities in green and woody attributes. CO27, on the negative x-axis, did not covary well with any sensory attributes. This was also in agreement with the sensory evaluation results, where CO27 did not have highest score in five attributes. CO55 and CO3 were located in the upper right hand quadrant, and correlated to propanoic acid (C2) and 6-methyl-5-hepten-2-one (D5). Pungent attribute located in the upper right hand quadrant was associated with pentanal (A1), octanol (B10), acetic acid (C1),

pentanoic acid (C3). Pentanoic acid (C3) showed significant associated to pungent in the coefficient of PLS regression model of pungent. Hexanal (A3), (*E*)-2-hexenal (A7), octanal (A8), (*E*)-2-heptenal (A9), nonanal (A10) showed a significantly positive contribution to green aroma. (*E*)-2-octenal (A11), (*E*)-2-decenal (A16), (*E*, *E*)-2, 4-heptadienal (A17), 2-hexanol (B5), 2-heptanol (B6), hexanol (B7), 1-*p*-menthene (E1) were highly associated to the woody attribute by means of the regression coefficients in the PLS regression model of woody. From the above results, it was revealed that most aroma compounds characterized by GC/MS made a great contribution to Camellia seed oil.

E-nose analysis of different Camellia seed oils E-nose combined with the principal component analysis technique was used to differentiate different Camellia seed oils. As shown in Fig.3, the variances explained by the first two principal components are 84.94% and 12.55% for PC1 and PC2, respectively. The same sample with repeated tests was located close to one another as a group/cluster and the differences between groups distributed different locations on the two-dimensional plane.

As shown in Fig. 3, each variety group was clearly distinguished from the other groups, and there were obvious difference among the oil samples. CO18 was located in the positive

Fig. 3. Two-dimensional (2D) PCA plot of the first two principal components based on the electronic nose (Codes in Table 1).

dimension of x axis, while CO21 in the opposite area. They were clearly distinct and separated from the others by PC1, which was consistent with the sensory analysis and GC/MS analysis. In addition, CO27, CO3 and CO55 were distributed in the vicinity of y-axis. The results indicated that the e-nose combined with PCA may be used to distinguish between samples from different varieties.

Conclusions

Aroma compounds in oils from different *Camellia oleifera* Abel. varieties were evaluated by combining HS-SPME/GC/MS and e-nose techniques with multivariate statistical analysis. PLSR revealed that 23 aroma compounds, pentanal, hexanal, (*E*)-2-hexenal, octanal, (*E*)-2-heptenal, nonanal, (*E*)-2-octenal, (*E*)-2-decenal, (*E,E*)-2,4-heptadienal, 2-hexanol, 2-heptanol, hexanol, octanol, acetic acid, pentanoic acid, 1-*p*-menthene, limonene, γ -terpinene, ethyl-2-methylbutanoate, 2-methylbutyl acetate, ethyl 2-methyl-2-butenate, γ -butyrolactone, γ -hexalactone, showed good coordination with *Camellia* seed oil characteristics. The consistency among GC/MS, sensory evaluation and e-nose analysis suggesting that e-nose technique combined with PCA has the good potential to classify *Camellia* seed oil samples from different varieties and the oil quality. In order to validate fully the usefulness of an e-nose for the classification of *Camellia* seed oil varieties, larger data will be involved in the next study. A better comprehension of this knowledge will be significantly useful for distinguishing the characteristic aroma of *Camellia* seed oils.

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