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Characterization and Authentication of Significant Chinese Edible Oilseed Oils by Stable Carbon Isotope Analysis

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Abstract The ratios of stable carbon isotopes ($\delta^{13}C$) of 12 oils extracted from Chinese edible oilseed samples and their individual fatty acids were determined by elemental analysis-isotope ratio mass spectrometry (EA-IRMS) and gas chromatography-isotope ratio mass spectrometry (GC-IRMS). The results have demonstrated that the $\delta^{13}C$ ratios of the oils from C3-plant seeds range from −26.8 to −30.7‰, while the $\delta^{13}C$ ratios of C4-plant maize oil are in the interval of −14.1 to −16.2‰. Eighteen fatty acids were identified and their abundances were measured by gas chromatography–mass spectrometry (GC–MS) in these oils with $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ as the major constituents. From the data on fatty acids and stable carbon isotopes, several sensitive markers were developed to detect the adulteration of Chinese edible oilseed oils. Examples are provided with pre-blended samples to illustrate the discrimination procedures and corresponding sensitive markers with emphasis on camellia seed oil, flax seed oil and perilla seed oil.

Keywords Edible oilseed oils · Stable carbon isotope analysis · Fatty acids · Adulteration

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Introduction

Oils and fats are one of the most important food classes in our daily life. Edible oilseed oils, containing more pleasant flavors and increased nutritional benefits, have recently become more popular than animal fats [1]. With the increasing demand for edible oilseed oils, their adulteration is quite prevalent all over the world. The adulteration modes have become more sophisticated in recent years, and the traditional discrimination methods are becoming more expensive and time-consuming as adulterators find ways of avoiding routine detection. Thus, diverse modern methods were developed in succession, such as Fourier transform infrared spectroscopy [2], chromatography [3, 4], mass spectrometry [5], nuclear magnetic resonance [6], electronic nose [7] and stable carbon isotope ratio analysis (SCIRA) [8].

SCIRA is a powerful technique for assessing the authenticity of vegetable food products from plants of different photosynthetic pathways [9]. The carbon isotope compositions of plants and their products are linked to the processes of photosynthetic atmospheric CO_2 fixation. During photosynthetic carbon fixation, plant cells discriminate against the heavier stable carbon isotope ^{13}C. The most important atmospheric CO_2-fixing reactions are C_3 and C_4 pathways. C_3 plants use the Calvin cycle to fix CO_2 and their carbon isotope compositions fall into the range of −22 to −34‰. C_4 plants adopt the Hatch–Stack cycle for CO_2 fixation, and produce much less isotopic fractionation with the heavier carbon isotope compositions (−17 to −9‰) [4, 10]. Most plants in the tropics, mainly including maize and sugar cane, are C_4 plants. In addition, environmental factors, such as light, water, salinity and air pollution, may also affect the carbon isotopic compositions of plants to a certain extent [10].

SCIRA is also potentially a powerful method to detect the adulteration of edible oils. For example, Woodbury et al. [11,

12] detected the adulteration of maize germ oil with C3-plant oils down to a level of 5%. In their further work, they determined the $\delta^{13}C$ values of the major fatty acids in more than 150 vegetable oils and established a database that provided isotopic information for authenticity control of vegetable oils. Kelly et al. [8] found that the $\delta^{13}C$ values for the authentic vegetable oil fatty acids fell within a narrow range of −27.6 to −32.1‰. Employing discriminant factor analysis, the $\delta^{13}C$ data for sunflower oil could be distinguished from other oils. Angerosa et al. [13] found the $\delta^{13}C$ values of the adulterant olive–pomace oil to be significantly more negative than those of virgin and refined olive oils, and employed both $\delta^{13}C$ and $\delta^{18}O$ analyses to determine the geographical origin of olive oils. Recently, our group developed two methods to discriminate whether *Ganoderma* spore lipid was adulterated with edible vegetable oils [14, 15]. Although stable carbon isotope databases of edible oils have been established in several countries [4, 12, 16], those databases cannot be directly adopted to determine the adulteration of Chinese edible oils from plant sources because of the close relationship of stable carbon isotope identities to geographical origins. There is now an urgent need to obtain the numerous high-quality data, and further establish the stable carbon isotope database of Chinese oilseed oils. In particular, Chinese agricultural products, including edible and speciality oils are being exported to many countries after China became a member of the World Trade Organization. Therefore, both China and the countries importing the Chinese edible oils require such a database to evaluate the quality of Chinese edible oils.

The most frequent adulteration of edible oilseed oils in China is that of camellia seed oil, perilla seed oil and flax seed oil, which are often adulterated with maize oil or soybean oil. Chinese organizations for public health inspection and supervision usually use the routine methods to discriminate the adulteration of oilseed oils. To our knowledge, the stable carbon isotope data of camellia seed oil, perilla seed oil, flax seed oil and pine nut oil have not been presented so far. Moreover, the stable carbon isotope data on Chinese edible oils are also scarce. In this paper, we first characterize 12 major Chinese edible oils for their fatty acid composition, and stable carbon isotopes of oils and their major fatty acid constituents; and then discuss the potential sensitive markers and discrimination procedures to evaluate their quality with emphasis on camellia seed oil, flax seed oil and perilla seed oil.

Experimental Procedure

Sample Description

Twelve samples, including camellia seed (Cam), perilla seed (Per), flax seed (Flax), maize seed (Maize), peanut (Pea), sesame (Ses), pine nut (Pine), pumpkin seed (PumA and PumB), sunflower seed (Sun), soybean (Soy) and walnut (Wal) of dry oilseeds produced in China were purchased for this study. These samples were manually milled with a domestic grinder, and were Soxhlet-extracted with petroleum ether (boiling point 30–60 °C) for 4 h. The suspension was filtered, and the residue was re-extracted with dichloromethane for another two times. The filtrates were combined, and the solvent was removed with a rotary evaporator at 40 °C under nitrogen flow. The details of these samples and their oil yields are presented in Table 1.

Reagents and Treatment

The solvents, petroleum ether (boiling point 30–60 °C), tetrahydrofuran, acetic acid, ethyl ether and ethyl acetate, and the reagents, anhydrous sodium sulfate and sulfuric acid were of analytical grade purchased from Chinese suppliers (GCRF, Guangzhou, China). Petroleum ether was purified by washing with concentrated sulfuric acid, 2% sodium carbonate solution and water, drying over anhydrous sodium sulfate and distilling with a rectifying column. Tetrahydrofuran, ethyl ether and ethyl acetate were purified by distilling with a rectifying column. Anhydrous petroleum ether, methanol (HPLC grade, Merck, Darmstadt, Germany) and tetrahydrofuran were obtained by refluxing the solvents with sodium wires followed by re-distillation, and the stable carbon isotope ratio of anhydrous methanol was determined separately by gas chromatography-isotope ratio mass spectrometry (GC-IRMS). Sodium methoxide (5%) was prepared by adding calculated amount of fresh sodium wires to anhydrous methanol.

Transesterification

Freshly prepared anhydrous tetrahydrofuran (1 mL) and sodium methoxide (5%, 1 mL) were added to a solution of ca. 50 mg lipid in 2 mL anhydrous petroleum ether (30–60 °C) in a penetrable screw capped vial with a dry syringe. After shaking and standing for 10 min, the mixture was neutralized with acetic acid (5%, 1 mL) and washed three times with distilled water. The petroleum ether layer containing fatty acid methyl esters (FAMEs) was dried with anhydrous sodium sulfate and filtered.

Gas Chromatography/Mass Spectrometry (Gc/Ms)

Analysis of the fatty acid methyl esters in 4 mL petroleum ether were achieved with a Varian GC CP-3800 gas chromatograph (Varian, Palo Alto, USA) equipped with a flame ionization detector and a capillary column (50 m length × 0.25 mm i.d., 0.25 μm film thickness, CP-7419, Varian, Palo Alto, USA). The carrier gas was helium at a

Table 1 Relative abundances (% of total fatty acids) of fatty acids in the oils extracted from 12 edible oilseeds

Sample[a]	Cam	Per	Flax	Maize	Pea	Pine	Pum A	Pum B	Ses	Sun	Soy	Wal
Content (%)	43.39	40.50	41.20	3.94	38.20	63.90	47.90	48.60	49.00	57.20	18.20	63.50
$C_{12:0}$	ND[b]	ND	ND	0.02	0.01	ND	0.01	0.01	0.01	ND	ND	ND
$C_{14:0}$	0.06	0.04	0.05	0.01	0.04	0.02	0.2	0.18	0.02	0.09	0.14	0.01
$C_{15:0}$	0.01	ND	0.02	0.01	0.01	0.01	0.03	0.01	ND	0.02	0.03	0.03
$C_{16:0}$	8.17	7.91	7.36	12.1	10.9	5.15	14.3	13.6	8.79	6.53	11.6	5.80
$C_{16:1}$	0.43	0.28	0.06	0.06	0.07	0.14	0.39	0.22	0.39	0.16	0.24	0.28
$C_{16:2}$	ND	ND	ND	ND	ND	0.01	ND	0.01	0.02	ND	0.02	0.06
$C_{17:0}$	0.10	0.11	0.14	ND	0.13	0.06	0.24	0.11	0.06	0.08	0.23	0.07
$C_{17:1}$	0.09	0.19	0.06	ND	0.04	0.06	0.07	0.04	0.02	0.07	0.06	0.03
$C_{18:0}$	2.11	2.49	3.83	2.73	6.08	2.57	6.98	5.29	6.57	6.78	5.69	3.26
$C_{18:1}$	79.5	19.8	27.1	28.6	42.2	27.7	25.9	34.4	37.4	22.3	23.3	28.3
$C_{18:2}$	8.67	13.9	14.7	55.6	35.6	45.6	50.9	45.0	39.8	62.1	47.0	53.0
$C_{18:3}$	0.06	54.8	46.0	ND	ND	14.3	0.01	ND	4.72	ND	11.0	8.96
$C_{20:0}$	0.02	0.11	0.17	0.58	2.05	0.48	0.65	0.55	1.00	0.46	0.23	0.03
$C_{20:1}$	0.76	0.11	ND	0.15	0.83	1.60	0.13	0.23	0.88	0.24	0.14	0.17
$C_{20:2}$	ND	ND	ND	ND	ND	0.77	0.01	0.10	0.20	0.05	0.08	ND
$C_{20:3}$	ND	ND	ND	ND	ND	0.99	ND	ND	ND	0.02	ND	ND
$C_{22:0}$	ND	0.16	0.44	0.15	1.92	0.36	0.14	0.18	0.17	1.06	0.39	0.02
$C_{22:1}$	0.03	ND	0.15	ND	0.15	0.10	ND	0.02	ND	ND	ND	ND
$C_{18:1}/C_{18:2}$	9.17	1.42	1.85	0.51	1.18	0.61	0.51	0.76	0.94	0.36	0.50	0.53
$C_{18:1}/C_{16:0}$	9.73	2.50	3.68	2.36	3.87	5.38	1.81	2.53	4.25	3.42	2.01	4.88
$C_{18:3}/C_{18:2}$	0.01	3.93	3.14	ND	ND	0.31	ND	ND	0.12	ND	0.23	0.17
SFA[c]	10.39	10.24	11.24	15.44	20.82	8.65	22.48	19.92	16.62	14.96	18.05	9.26
UFA[c]	89.61	89.76	88.76	84.56	79.18	91.35	77.52	80.08	83.38	85.04	81.95	90.74
UFA/SFA	8.62	8.77	7.90	5.48	3.80	10.56	3.45	4.02	5.02	5.68	4.54	9.80

The data are the means of three determinations, and have typical standard deviations of less than 2.3% ($SD_{C16:0} \leq 0.20\%$; $SD_{C18:0} \leq 0.05\%$; $SD_{C18:1} \leq 2.21\%$; $SD_{C18:2} \leq 1.70\%$; $SD_{C18:3} \leq 1.46\%$; and the SDs of minor fatty acids less than 0.05%). Camellia seed, perilla seed and flax seed were produced in Jiangxi, Guizhou and Inner Mongolia, respectively; maize, peanut, sesame and sunflower seed were produced in Guangdong; pumpkin seed A and B were produced in Yangjiang and Guangzhou, respectively; and pine nut, soybean and walnut were produced in northern China

[a] Cam, Per, Flax, Maize, Pea, Pine, Pum A, Pum B, Ses, Sun, Soy and Wal represent camellia seed oil, perilla seed oil, flax seed oil, maize oil, peanut oil, pine nut oil, pumpkin seed oil A, pumpkin seed oil B, sesame seed oil, sunflower seed oil, soybean oil and walnut oil, respectively

[b] ND, not detected

[c] SFA and [c]UFA are the relative abundances of saturated, and unsaturated fatty acids, respectively

constant flow rate of 1.0 mL/min, and injection was in the split mode with a split ratio of 10:1. The following temperature program was used: initial oven temperature 100 °C, held for 2 min; first program rate, 6 °C/min to 190 °C and held 10 min; second program rate, 20 °C/min to 280 °C, and held for 5 min. Individual FAME was identified by comparison with an external FAME standard (FAME Mix C4-C24, Sigma–Aldrich, St. Louis, MO, USA). FAMEs were also analyzed by GC–MS with a GC (CP-3800, Varian, Palo Alto) and a ion trap mass spectrometer (Saturn 2000, Varian, Palo Alto). Identification of individual compounds was achieved by comparing the mass spectrum with that of the library spectrum. The conditions of mass spectrometer analysis were as follows: electron ionization (EI) with a voltage of 70 eV, the source temperature 250 °C, and the mass scan range from 40 to 600. Before sampling, blank runs were performed to ensure that there was no carryover of analytes from previous extractions. All of the samples were analyzed in triplicate, and the standard deviations were less than 2.3%.

Gas Chromatography-Isotope Ratio Mass Spectrometry

Stable carbon isotope measurements of individual FAMEs were carried out on an HP 6890 gas chromatograph equipped with a split/splitless injector interfaced via a combustion furnace to an Isoprime isotope ratio mass spectrometer (GV IsoPrime, GV Instruments, Manchester, UK). The combustion interface contained Cu wires, which were doped with oxygen (CuO) and maintained at a temperature of 960 °C.

Samples were injected into the GC-IRMS system with a splitless mode by a stream of helium carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was maintained at 290 °C. The separation of compounds was performed on an identical column to that described above and used the same temperature program. The mass spectrometer measured the relative abundances of the *m/z* 44 ($^{12}CO_2$) and *m/z* 45 ions ($^{13}CO_2$). The contribution of $^{12}C^{17}O^{16}O$ to mass 45 was automatically corrected by the instrument according to Craig's correction factor [17] and the measured ^{18}O value at *m/z* 46. The system was calibrated against a CO_2 reference gas with the known isotopic composition introduced into the isotopic ratio mass spectrometer in a series of pulses at the beginning and end of each analysis. The corrections were made by comparison with the CO_2 reference gas as an internal standard. All isotope values were expressed by the conventional delta (δ) notation:

$$\delta^{13}C_{PDB}(‰) = \left[\left(R_{sample}/R_{std}\right) - 1\right] \times 10^3 \quad (1)$$

where R_{sample} and R_{std} are the $^{13}C/^{12}C$ isotope ratios corresponding to the sample and international conventional V-PDB (Vienna Pee Dee Belemnite) carbonate internal standard, respectively [17]. The deviation for the detected $\delta^{13}C$ value (‰) of this CO_2 reference gas was typically less than ±0.1‰, and showed good stability of the instrument during a complete GC run. In addition, a standard mixture containing ten *n*-alkanes with known isotopic compositions was analyzed two or three times per day in order to check the precision and reproducibility of the instrument. The detected deviation from the actual value for the *n*-alkanes standard sample was typically less than ±0.5‰. Three parallel analyses were also performed on our samples. The standard deviations for FAMEs were mostly in the range of 0.15–0.26‰ and showed good reproducibility.

Because each FAME contained a methyl group from the trans-esterification that was not present in the original molecule, it was necessary to correct the $\delta^{13}C$ value of each FAME. The $\delta^{13}C$ value of the methyl group was determined by measuring the $\delta^{13}C$ value of the methanol reagent used to methylate the acids. The $\delta^{13}C$ value (mean ± standard deviation) of the methyl group was −47.0 ± 0.15‰ (six replicate analyses). With a known $\delta^{13}C$ value for the methyl group, the $\delta^{13}C$ values of the original fatty acids of all samples were calculated as following:

$$\delta^{13}C_{FA} = [(n+1)\,\delta^{13}C_{FAME} - \delta^{13}C_{Methylgroup}]/n \quad (2)$$

where FA represents fatty acids and *n* is the number of carbon atoms in the fatty acid. $\delta^{13}C_{FAME}$ and $\delta^{13}C_{Methyl\ group}$ represent the measured $\delta^{13}C$ values of the FAME and its corresponding methanol reagent for esterification, respectively. The standard deviations of the calculated $\delta^{13}C$ values of the original fatty acids were chiefly in the range of 0.15–0.27‰.

Elemental Analysis-Isotope Ratio Mass Spectrometry

To determine the $\delta^{13}C$ values of oils, ca. 2 mg of each sample was weighed and loaded into a clean tin capsule. The capsules containing samples were placed in a CE EA1112 C/N/S analyzer (CE Instruments, Wigan, UK) and burned in an O_2 atmosphere in the combustion CuO tube with its temperature set at 960 °C. Combustion gases were eluted through a reduction column by a stream of He gas and passed into the gas chromatograph where CO_2, still in the He stream, was separated from the other gases. The gas stream then entered a Delta^{plus}XL mass spectrometer (Finnigan, Thermo Scientific, Waltham, MA, USA) where the CO_2 gas was analyzed compared to the reference CO_2 gas of a known $\delta^{13}C$ value (−29.1‰, calibrated against the NBS-22 reference material with a $\delta^{13}C$ value of –29.7‰). During every batch of analyses, an empty tin capsule was analyzed as the blank to check the background, and a carbon black sample of the known $\delta^{13}C$ value (–36.9‰) was used to check the reproducibility and accuracy. The corresponding standard deviation of analysis and the deviation between the measured data and pre-determined data were less than 0.3‰. Low background (peak height <0.02 V, much lower than the peak height of the sample, greater than 1.5 V) and excellent reproducibility and accuracy were achieved.

Results and Discussion

Yields and Basic Properties of Oils

The contents and basic properties of extracted oils are presented in Table 1. It is shown that the oil yields of different oilseeds are evidently different, ranging from 3.94 to 63.90% of the oilseeds on a dry weight basis. The oil yields of pine nut, walnut and sunflower seed are higher than those of the other oilseeds, ranging from 63.90 to 57.20%. Sesame seed, pumpkin seed, camellia seed, flax seed, perilla seed and peanut have higher oil yields, ranging from 49.00 to 38.20%. The oil yields of soy bean and maize are down to the levels of 18.20 and 3.94%, respectively (Table 1).

Variation of Major Fatty Acid Abundances

Eighteen fatty acids were distinguished from the 12 oilseed oils by GC/MS, and their relative abundances of fatty acids in all studied samples, calculated from the peak areas of chromatograms and are presented in Table 1. It can be seen from Table 1 that $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ are major fatty acids. $C_{18:2}$ was determined to be the predominant fatty acid in the interval of 39.8–62.1%, and

$C_{18:1}$ was measured to be the second abundant fatty acid between 22.3 and 37.4% in most of the oil samples except camellia seed oil, flax seed oil, perilla seed oil and peanut oil. $C_{18:1}$ is the fatty acid with the highest abundance in camellia seed oil (79.5%) and peanut oil (42.2%). $C_{18:3}$ is the dominant fatty acid in perilla seed oil (54.8%) and flaxseed oil (46.0%), whereas the abundance of $C_{18:3}$ is low in camellia seed oil, maize oil, peanut oil, pumpkin seed oil and sunflower seed oil, and sesame oil, walnut oil, soybean oil and pine nut oil contain a small quantity of $C_{18:3}$, ranging from 4.72 to 14.3%. The abundances of $C_{16:0}$ and $C_{18:0}$ are relatively low in all samples, ranging from 5.15 to 14.3 and 2.11 to 6.98%, respectively. The abundance of $C_{16:0}$ is highest in the two pumpkin seed oils (14.3 and 13.6%) and lowest in the pine nut oil (5.15%). Our data of the fatty acids for camellia oil, perilla oil and flax seed oil are similar to previously-published results [2, 3, 18].

Stable Carbon Isotopic Composition of Bulk Oils

The $\delta^{13}C$ values of 12 bulk oil samples determined by elemental analysis-isotope ratio mass spectrometry (EA-IRMS) are shown in Table 2. The results show that the bulk maize oil appears to have the specific stable carbon isotope composition of C4 plants with a $\delta^{13}C$ value of –14.1‰ [19], while the others have the $\delta^{13}C$ values of –30.1 to –26.8‰, showing the typical stable carbon isotope composition of C3 plants. Obviously, it is easy to discriminate the adulteration between the edible oils of C3 and C4 plants, due to their remarkable difference in stable carbon isotope composition.

Table 2 $\delta^{13}C$ values (‰) of bulk oils and their individual major fatty acids in the oils extracted from 12 edible oilseeds

	$\delta^{13}C$ value (‰)				
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	Bulk
Cam	−28.8	−29.4	−28.2	−28.7	−28.6
Per	−28.7	−29.8	−27.8	−27.5	−28.5
Flax	−30.1	−30.5	−29.2	−29.7	−29.4
Maize	−15.3	−16.2	−15.1	−15.0	−14.1
Pea	−28.4	−29.1	−27.5	−27.3	−28.1
Pine	−26.9	−28.2	−26.9	−27.6	−26.8
Pum A	−28.8	−29.5	−28.5	−28.6	−27.6
Pum B	−29.1	−29.5	−28.1	−28.6	−28.0
Ses	−29.7	−30.7	−29.2	−29.1	−28.8
Sun	−29.2	−29.5	−28.7	−28.5	−28.4
Soy	−30.0	−30.6	−29.8	−29.7	−30.1
Wal	−28.5	−29.1	−27.9	−28.2	−27.4

The $\delta^{13}C$ values are the means of three determinations, and have their typical standard deviations less than 0.3‰ ($SD_{C16:0} \leq 0.15$‰; $SD_{C18:0} \leq 0.27$‰; $SD_{C18:1} \leq 0.19$‰; $SD_{C18:2} \leq 0.23$‰; and $SD_{bulk} \leq 0.3$‰)

The $\delta^{13}C$ ratios of pine nut oil, walnut oil and pumpkin seed oil A are nearly equal to –27.0‰ (–26.8, –27.4 and –27.6‰); the $\delta^{13}C$ ratios of pumpkin seed oil B, peanut oil, sunflower seed oil, perilla seed oil, camellia seed oil and sesame seed oil are distributed in a narrow interval of –28.0 to –29.0‰; and the $\delta^{13}C$ ratios of flax seed oil and soybean oil are lower than –29.0‰ (–29.4 and –30.1‰). The $\delta^{13}C$ scatter of these C3 oils (3.3‰) may result from the stable carbon isotope effect in the fixation of carbon dioxide. This shift of stable carbon isotopes may be partially explained

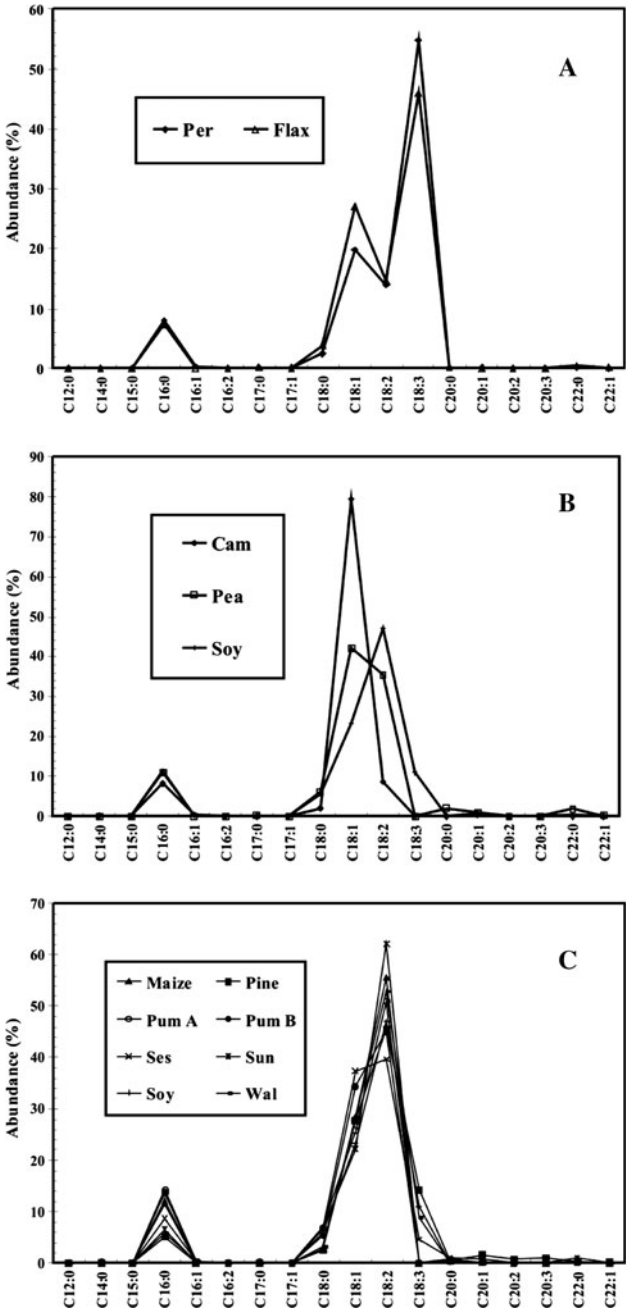

Fig. 1 Fatty acids versus abundances diagram, illustrating the fatty acid profiles of the oils extracted from 12 Chinese edible oilseeds

by the factors affecting the chemical distribution of fatty acids, particularly the physiological process and enzymatic reactions occurring in plant cells [20]. It is also demonstrated that the above $\delta^{13}C$ values for most oils of C3 plants are similar to existing data, but the $\delta^{13}C$ values of bulk samples of sesame seed oil (–28.8‰), peanut oil (–28.1‰) and pumpkin seed oil (–28.0 and –27.6‰) are evidently different from those published data of –26.5 to –25.1, –26.8 and –29.1 to –28.7‰ for the corresponding oils produced in other countries [4, 12, 16]. These newly-obtained data further suggest that it is quite necessary to establish a stable carbon isotope database of Chinese edible oils for authenticating their adulteration. Simultaneously, the variation of $\delta^{13}C$ values among sesame seed oils may be attributed to different geographical origin, different variety, growth conditions and harvest time [12]. The $\delta^{13}C$ difference between two pumpkin seed oils may also be attributed to their different growth circumstances [21]. The $\delta^{13}C$ values of pine nut oil (–26.8‰) and walnut oil (–27.4‰) are evidently higher than those of other oils of C3 plants, suggesting that it may be related to perennial woody plants.

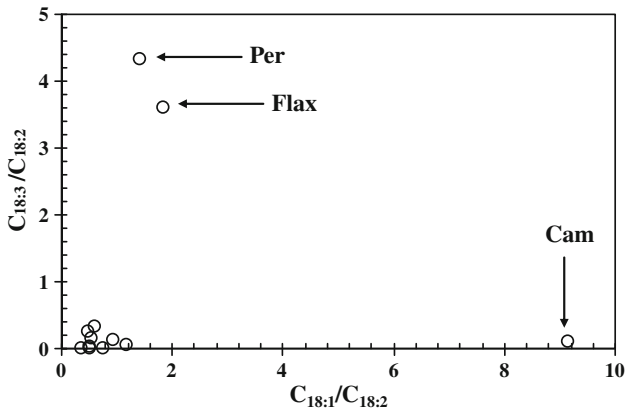

Fig. 2 $C_{18:1}/C_{18:2}$ versus $C_{18:3}/C_{18:2}$ plot, showing the evident difference in $C_{18:X}$ fatty acids among (Per + Flax), Cam and the other oils

Stable Carbon Isotope Composition of Major Fatty Acids

The $\delta^{13}C$ values of individual major fatty acids in the 12 oils determined by GC-IRMS are presented in Table 2. The $\delta^{13}C$ ratios of $C_{16:0}$ of the oils except maize oil range from –26.9 to –30.1‰, and most of them fluctuate around –29.0‰. The $\delta^{13}C$ values of $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ of the oils except maize oil are in the intervals of –28.2 to –30.7, –26.9 to –29.8 and –27.3 to –29.7‰, respectively. The differences in $\delta^{13}C$ values between $C_{18:0}$ and $C_{16:0}$, $C_{18:1}$ and $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$, and $C_{18:1}$ and $C_{16:0}$ range from 0.3 to 1.3, 0.0 to 1.1, −0.62 to 0.31, and 0.8 to 2.0‰, and have average values of 0.7, 0.5, −0.1 and 1.3‰, respectively. The result also shows that the $\delta^{13}C$ value decreased slightly (0.7‰) in the elongation from $C_{16:0}$ to $C_{18:0}$, increased significantly (1.3‰) in the first de-saturation from $C_{18:0}$ to $C_{18:1}$, and kept stable in the further de-saturation from $C_{18:1}$ to $C_{18:2}$, displaying a similar trend, i.e., $\delta^{13}C_{18:0} < \delta^{13}C_{16:0} < \delta^{13}C_{18:1} \approx \delta^{13}C_{18:2}$. This uniform trend may suggest that the biosynthetic reactions of major

Table 3 Relative abundances of major fatty acids in ideal blending samples

	$C_{16:0}$	$C_{18:2}$	$C_{18:3}$	$C_{18:1}$	$C_{18:0}$	$C_{18:1}/C_{18:2}$	$C_{18:3}/C_{18:2}$
90% Cam + 10% Soy	8.51	12.50	1.15	73.87	2.47	5.91	
70% Cam + 30% Soy	9.18	20.15	3.33	62.63	3.19	3.11	
50% Cam + 50% Soy	9.86	27.81	5.51	51.39	3.90	1.85	
90% Cam + 10% Maize	8.65	12.49	0.05	75.15	2.20	6.02	
70% Cam + 30% Maize	9.44	21.97	0.04	64.87	2.32	2.95	
50% Cam + 50% Maize	10.24	31.46	0.03	54.58	2.45	1.74	
90% Per + 10% Soy	8.28	17.24	50.42	20.18	2.81		2.93
70% Per + 30% Soy	9.01	23.84	41.65	20.87	3.45		1.75
50% Per + 50% Soy	9.73	30.45	32.88	21.56	4.09		1.08
90% Per + 10% Maize	8.42	17.28	49.82	20.92	2.54		2.88
70% Per + 30% Maize	9.26	25.70	38.75	22.69	2.59		1.51
50% Per + 50% Maize	10.11	34.12	27.68	24.45	2.64		0.81
90% Flax + 10% Soy	7.78	17.89	42.48	26.70	4.01		2.38
70% Flax + 30% Soy	8.61	24.35	35.48	25.94	4.39		1.46
50% Flax + 50% Soy	9.45	30.81	28.47	25.19	4.76		0.92
90% Flax + 10% Maize	7.91	17.94	41.80	27.51	3.75		2.33
70% Flax + 30% Maize	8.87	26.21	32.51	27.81	3.53		1.24
50% Flax + 50% Maize	9.82	34.49	23.22	28.11	3.31		0.67

fatty acids are essentially identical in these studied plants, and can be rationally explained by multi-enzyme complex catalysis [4]. The huge difference in the $\delta^{13}C$ ratios of individual major fatty acids occurs between maize oil and the other oils, i.e., much more enriched ^{13}C in the maize oil with the $\delta^{13}C$ ratios from –15.0 to –16.2‰.

Authentication of Chinese Edible Oilseed Oils

Camellia seed oil, flax seed oil and perilla seed oil are rare and valuable health foods exhibiting specific bioactivities. Economic incentives induce manufacturers to adulterate them with cheaper edible oils. However, it is often difficult to detect this kind of adulteration, due to uncertainties associated with existing methods of analysis of oils and bioactive ingredients. For example, the adulteration detection of the above three oils by determination of fatty acid composition is limited by the fact that fatty acids overlap in the other edible oils. Furthermore, blending of cheaper edible oils can yield compositions similar to those oils. Thus, it is necessary to search for sensitive markers such as the pattern of stable carbon isotopes combined with fatty acid composition to discriminate the adulteration of these rare oils.

Because C4-plant oils typically have $\delta^{13}C$ values (‰) between –9 and –17‰ [4, 10], the adulteration of camellia seed oil, perilla seed oil and flax seed oil with C4-plant oils can be easily determined by EA-IRMS and GC-IRMS. However, the $\delta^{13}C$ values (‰) from C3-plant oils were lower at the interval of –22 to –34‰ [4, 10], overlapping with those of camellia seed oil, perilla seed oil and flax seed oil (Table 2). Hence, the $\delta^{13}C$ values (‰) of oils and their fatty acids are insufficient to discriminate unambiguously the adulteration of camellia seed oil, perilla seed oil and flax seed oil with C3-plant oils. From their fatty acid composition and stable carbon isotopes, we propose an integrated method to detect the adulteration of camellia seed oil, perilla seed oil and flax seed oil with C4- or/and C3-plant oils, and give several pre-blended oil examples to elucidate the discrimination procedures and markers.

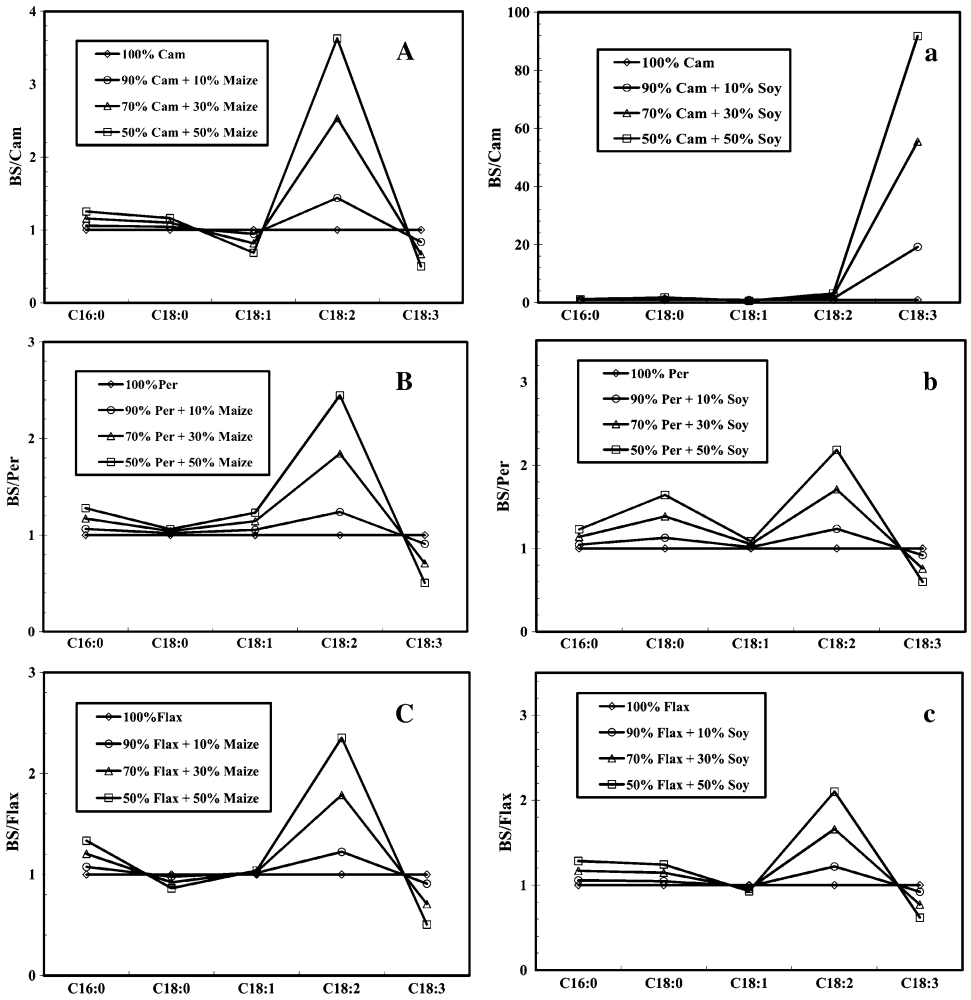

Fig. 3 Cam-normalized (**A**, **a**), Per-normalized (**B**, **b**) or Flax-normalized (**C**, **c**) patterns of major fatty acids in the pre-blended oil samples adulterated with maize oil (**A**, **B**, **C**) or soybean oil (**a**–**c**) (BS denotes pre-blended oil)

Step 1: Fatty Acid Profiles

The fatty acid profiles of the tested oils are presented in Fig. 1. It can be seen that the fatty acid profiles may be subdivided into three types. Type 1 is represented by perilla seed oil and flax seed oil, and characterized by two peaks at $C_{16:0}$ and $C_{18:3}$ and relatively high contents of $C_{18:1}$ and $C_{18:2}$. $C_{18:3}$ is abundant, up to 46.0% (flax seed oil) and 54.8% (perilla seed oil) (Table 1, Fig. 1A). Type 2 is represented by camellia seed oil and peanut oil, and characterized by three peaks at $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$, but the peak height of $C_{18:1}$ much higher than that of $C_{18:2}$, and the peak height of $C_{18:1}$ higher than that of $C_{16:0}$ (Fig. 1B). The rest of the oils belong to type 3, and also characterized by three peaks at $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$, but the peak height of $C_{18:1}$ evidently lower than that of $C_{18:2}$ and the peak height of $C_{18:1}$ higher than that of $C_{16:0}$ (Fig. 1C). These specific profiles may be used to detect the adulteration of edible oils, and roughly calculate the adulterant concentration.

If one commercial camellia seed oil has its fatty acid profile evidently different from that of camellia seed oil, e.g., the profile of soy. in Fig. 1B, it shows it to be adulterated with the other edible oils. Inversely, if its fatty acid profile is the same as that of camellia seed oil, e.g., the profile of Pea in Fig. 1B, the possibility of adulteration with the other edible oils can still not be excluded, due to their similarity between the camellia seed oil and adulterant oils.

Step 2: Ratios of $C_{18:1}/C_{18:2}$, $C_{18:1}/C_{16:0}$, $C_{18:3}/C_{18:2}$ and UFA/SFA

The abundances of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$, and their ratios of $C_{18:1}/C_{18:2}$, $C_{18:1}/C_{16:0}$ and $C_{18:3}/C_{18:2}$ are presented in Table 1. It shows that the abundances of $C_{18:1}$ in camellia seed oil and $C_{18:3}$ in perilla seed oil and flax seed oil are extraordinarily high, implying that the two fatty acids may be sensitive markers for detecting the adulteration of these oils. The $C_{18:1}/C_{18:2}$ and $C_{18:1}/C_{16:0}$ ratio of camellia oil (9.17 and 9.73, respectively) is much higher than those of most oils, the $C_{18:3}/C_{18:2}$ ratios of flax seed oil and perilla seed oil are higher than 3, and the others are less than 0.4 (Table 1). Thus, the ratios of $C_{18:1}/C_{18:2}$, $C_{18:1}/C_{16:0}$ and $C_{18:3}/C_{18:2}$ may also be sensitive markers to discriminate the adulterants in camellia seed oil, perilla seed oil and flax seed oil. Furthermore, the $C_{18:1}/C_{16:0}$ ratios of pine nut oil and walnut oil approach 5 and are comparatively higher than the others, the only exception being camellia oil, and may also be applied to the detection of adulterants.

It can be further seen in Fig. 2 that the studied oils may be divided into three clearly evident separate areas, i.e., most of the studied oils are concentrated in a narrow area close to the origin point, and perilla seed oil accompanied by flax seed oil but both of them are far from camellia seed oil. Obviously, Fig. 2 can be used to distinguish among (Per + Flax), Cam and the rest of the oils. Moreover, the adulterant concentration (C_A) may be calculated on the basis of the following equation:

$$C_A = (B - G)/(A - G) \quad (3)$$

where G, A and B are the ratios of $C_{18:1}/C_{18:2}$ ($C_{18:1}/C_{16:0}$ or $C_{18:3}/C_{18:2}$) in genuine oil, adulterant oil and pre-blended oil samples.

The contents of saturated fatty acids (SFA) and unsaturated fatty acids (UFA), and their ratios are presented in Table 1. It can be seen from Table 1 that pine nut oil and walnut oil have higher ratios of UFA/SFA (10.56 and 9.80); camellia seed oil, perilla seed oil and flax seed oil possess medium ratios of UFA/SFA even with a small variation (8.77–7.90); the ratios of the rest of the oils fluctuate around 4.50; and peanut oil and pumpkin seed oil A have the lowest ratios of UFA/SFA (3.80 and 3.45). Hence, the ratio of UFA/SFA may also be an important marker for detecting the adulteration of edible oils.

Step 3: Patterns of Major Fatty Acids

The abundances of major fatty acids in the pre-blended samples as well as the ratios of $C_{18:1}/C_{18:2}$ and $C_{18:3}/C_{18:2}$ are presented in Table 3. The Cam-, Per- and Flax-normalized plots of major fatty acids $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ are illustrated in Fig. 3. To describe the

Table 4 $\delta^{13}C$ ratios (‰) of the bulk oils and their individual major fatty acids in ideal blending samples

	$\delta^{13}C$ value (‰)				
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	Bulk oil
90% Cam + 10% Maize	−26.9	−27.7	−27.7	−23.0	−27.2
70% Cam + 30% Maize	−23.6	−24.7	−26.5	−18.7	−24.3
50% Cam + 50% Maize	−20.7	−20.9	−24.7	−16.9	−21.4
90% Per + 10% Maize	−26.8	−28.3	−26.1	−23.7	−27.1
70% Per + 30% Maize	−23.4	−25.5	−23.0	−19.7	−24.2
50% Per + 50% Maize	−20.6	−22.7	−20.3	−17.5	−21.3
90% Flax + 10% Maize	−27.8	−29.5	−27.7	−25.3	−27.9
70% Flax + 30% Maize	−24.0	−27.2	−24.8	−20.6	−24.8
50% Flax + 50% Maize	−20.9	−24.6	−22.0	−18.1	−21.8
90% Cam + 10% Soy	−29.0	−29.7	−28.3	−29.1	−28.8
70% Cam + 30% Soy	−29.3	−30.2	−28.4	−29.4	−29.1
50% Cam + 50% Soy	−29.5	−30.4	−28.6	−29.5	−29.4
90% Per + 10% Soy	−28.9	−30.0	−28.0	−28.1	−28.7
70% Per + 30% Soy	−29.2	−30.2	−28.5	−28.8	−29.0
50% Per + 50% Soy	−29.5	−30.4	−28.9	−29.2	−29.3
90% Flax + 10% Soy	−30.1	−30.5	−29.3	−29.7	−29.5
70% Flax + 30% Soy	−30.1	−30.5	−29.4	−29.7	−29.6
50% Flax + 50% Soy	−30.0	−30.6	−29.5	−29.7	−29.8

changes of these figures more intuitively and clearly, the data points are joined by lines to exhibit the specific patterns of major fatty acid profiles. It is shown that the curves of all patterns for one oil regularly glide up and down with the increase in the adulterant concentrations (Fig. 3). For the samples adulterated with maize oil, their curves slip more dramatically at $C_{18:2}$ due to the greater difference between Cam and Maize (Fig. 3A), Per and Maize (Fig. 3B) or Flax and Maize (Fig. 3C). However, for the samples adulterated with Soy, their curves depend on the kind of oils. The curves slip more dramatically at $C_{18:3}$ for Cam (Fig. 3a), $C_{18:0}$ and $C_{18:2}$ for Per (Fig. 3b), and $C_{18:2}$ for Flax (Fig. 3c). Therefore, for the adulterant maize oil, the positive anomaly at $C_{18:2}$, and for the adulterant soybean oil, the positive anomaly at $C_{18:3}$ (camellia seed oil), the positive anomalies at $C_{18:0}$ and $C_{18:2}$ (perilla seed oil) and the positive anomaly at $C_{18:2}$ (flax seed oil) may be employed as markers to observantly discriminate the adulteration of camellia seed oil, perilla seed oil and flax seed oil.

Step 4: δ^{13}c Patterns of Bulk Oils and Individual Major Fatty Acids

The compositions of stable carbon isotopes in the pre-blended samples are presented in Table 4, and the plots of Cam-, Per- and Flax-normalized δ^{13}C patterns are illustrated in Fig. 4. The data points were also connected by lines to visually exhibit the patterns of stable carbon isotopes. It is demonstrated that the curves of all patterns may glide down smoothly with notable slippages and an anomaly at $C_{18:2}$ for the samples adulterated with maize oil with the increase of the adulterant concentrations (Fig. 4A–C). However, the curves of patterns for the samples adulterated with soybean oil change more complicatedly and unevenly with the increase in the adulterant concentrations (Fig. 4a–c). All of the curves rise unevenly with no notable slippages but with evident anomalies for the samples adulterated with soybean oil with the increase in the adulterant concentrations (Fig. 4a–c). For camellia seed oil, the positive anomalies occur at $C_{18:0}$ and $C_{18:2}$ (Fig. 4a); for perilla seed oil, the

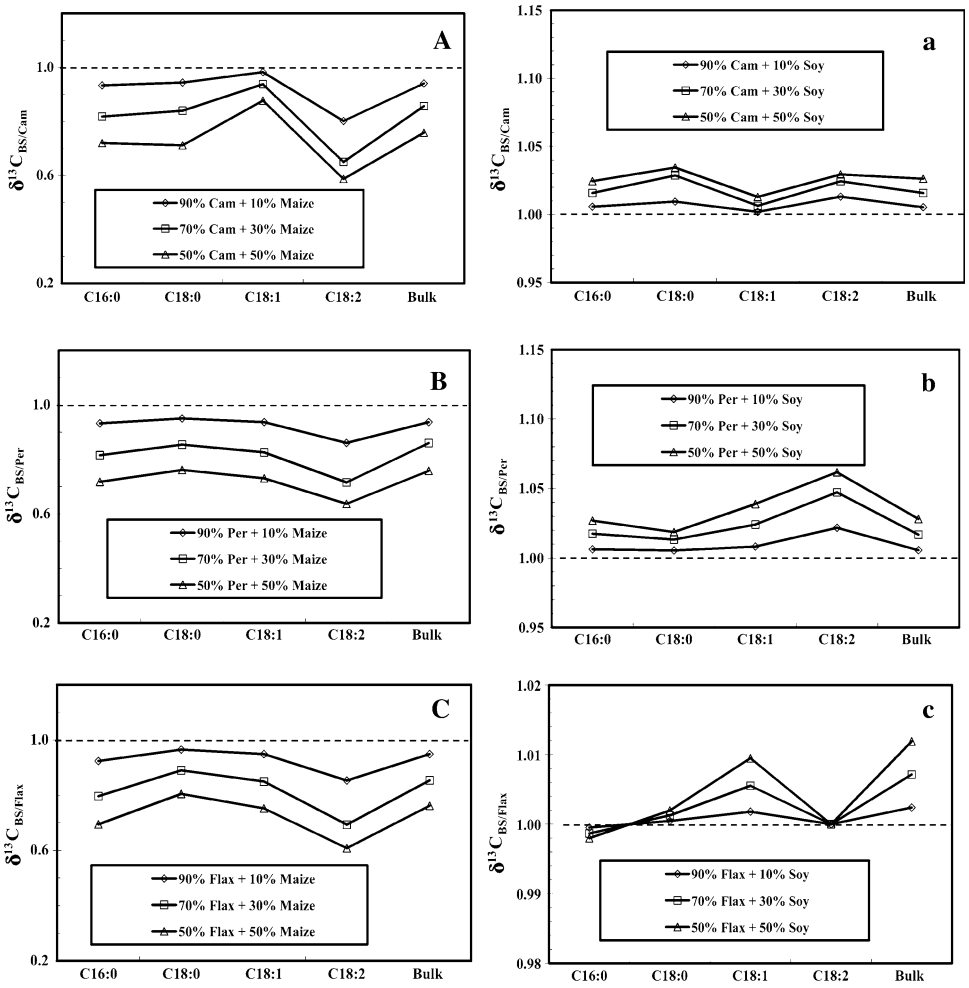

Fig. 4 Cam-normalized (**A**, **a**), Per-normalized (**B**, **b**) or Flax-normalized (**C**, **c**) δ^{13}C patterns of the pre-blended oil samples adulterated with maize oil (**A**, **B**, **C**) or soybean oil (**a**–**c**) and their major fatty acids (BS denotes pre-blended oil)

positive anomalies appear at $C_{18:1}$ and $C_{18:2}$ (Fig. 4b); and for flax seed oil, the positive anomalies occur at bulk and $C_{18:1}$ (Fig. 4c). Therefore, the above specific patterns combining with the anomalies and slippages may be sensitive markers for detecting the adulteration of camellia seed oil, perilla seed oil and flax seed oil with the adulterants maize oil or soybean oil.

The $\delta^{13}C$ value is a very sensitive marker for detecting adulterants if a C3-plant oil is adulterated with a C4-plant oil. Based on the analytical error of EA-IRMS ($\leq \pm 0.3‰$) in this study as well as the $\delta^{13}C$ values of C3-plant ($\delta^{13}C_{C3} = -34 \sim -22‰$) and C4-plant ($\delta^{13}C = -17 \sim -9‰$) oils from the published references [4, 10, 16], the adulterant concentration (C_A) can also be estimated from Eq. 3 with a detectable level down to 1.2–6.0%. When the $\delta^{13}C$ values of major fatty acids are adopted to calculate C_A, the relative abundances of fatty acids should take into consideration, and Eq. 3 may accordingly be altered [11].

It should be pointed out that the proposed method is quite suitable for detecting the adulteration between the oils from plants with different photosynthetic pathways, due to the remarkable differences between their $\delta^{13}C$ values, but not so feasible if the oils and adulterants possess similar isotopic signatures. It is also important to realize that it would be better to authenticate the adulteration of Chinese edible oils based on the databases of Chinese edible oils because of the differences in stable carbon isotope composition of edible oil sources between China and other countries as revealed in this study. Finally, it should be emphasized that in order to enhance the discernment and in particular avoid the misalignment of adulterants, the fatty acid profiles, ratios of $C_{18:1}/C_{18:2}$, $C_{18:1}/C_{16:0}$, $C_{18:3}/C_{18:2}$ and UFA/SFA, patterns of major fatty acids, $\delta^{13}C$ patterns of bulk oils and individual major fatty acids may be jointly considered as multiple indicators to detect the adulteration of camellia seed oil, perilla seed oil and flax seed oil with other cheaper edible vegetable oils.

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References

1. Aparicio R, Aparicio-Ruíz R (2000) Authentication of vegetable oils by chromatographic techniques. J Chromatogr A 881:93–104
2. Lazzari M, Chiantore O (1999) Drying and oxidative degradation of linseed oil. Polym Degrad Stab 65:303–313
3. Zhong HY, Bedgood DR Jr, Bishop AG, Prenzler PD, Robards K (2007) Endogenous biophenol, fatty acid and volatile profiles of selected oils. Food Chem 100:1544–1551
4. Spangenberg JE, Ogrinc N (2001) Authentication of vegetable oils by bulk and molecular carbon isotope analyses with emphasis on olive oil and pumpkin seed oil. J Agric Food Chem 49:1534–1540
5. Yang B, Kallio H (2006) Analysis of triacylglycerols of seeds and berries of sea buckthorn (*Hippophaë rhamnoides*) of different origins by mass spectrometry and tandem mass spectrometry. Lipids 41:381–392
6. Guillén MD, Ruiz A (2001) High resolution ^{1}H nuclear magnetic resonance in the study of edible oils and fats. Trends Food Sci Tech 12:328–338
7. Hai Z, Wang J (2006) Detection of adulteration in camellia seed oil and sesame oil using an electronic nose. Eur J Lipid Sci Tech 108:116–124
8. Kelly S, Parker I, Sharman M, Dennis J, Goodall I (1997) Assessing the authenticity of single seed vegetable oils using fatty acid stable carbon isotope ratios ($^{13}C/^{12}C$). Food Chem 59:181–186
9. Rossmann A, Koziet J, Martin GJ, Dennis MJ (1997) Determination of the carbon-13 content of sugars and pulp from fruit juices by isotope-ratio mass spectrometry (internal reference method): a European interlaboratory comparison. Anal Chim Acta 340:21–29
10. Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev 40:503–537
11. Woodbury SE, Evershed RP, Rossell JB, Griffith RE, Farnell P (1995) Detection of vegetable oil adulteration using gas chromatography combustion/isotope ratio mass spectrometry. Anal Chem 67:2685–2690
12. Woodbury SE, Evershed RP, Rossell JB (1998) $\delta^{13}C$ analyses of vegetable oil fatty acid components, determined by gas chromatography-combustion-isotope ratio mass spectrometry, after saponification or regiospecific hydrolysis. J Chromatogr A 805:249–257
13. Angerosa F, Bréas O, Contento S, Guillou C, Reniero F, Sada E (1999) Application of stable isotope ratio analysis to the characterization of the geographical origin of olive oils. J Agric Food Chem 47:1013–1017
14. Yuan JP, Wang JH, Liu X, Kuang HC, Huang XN (2006) Determination of ergosterol in ganoderma spore lipid from the germinating spores of *Ganoderma lucidum* by high-performance liquid chromatography. J Agric Food Chem 54:6172–6176
15. Liu X, Xu SP, Wang JH, Yuan JP, Guo LX, Li X, Huang XN (2007) Characterization of ganoderma spore lipid by stable carbon isotope analysis: implications for authentication. Anal Bioanal Chem 388:723–731
16. Benson S, Lennard C, Maynard P, Roux C (2006) Forensic applications of isotope ratio mass spectrometry: a review. Forensic Sci Int 157:1–22
17. Craig H (1957) Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim Cosmochim Acta 12:133–149
18. Salunkhe DK, Chavan JK, Adsule RN, Kadam SS (1999) World oilseeds: chemistry, technology and utilization. van Nostrand Reinhold, New York
19. O'Leary MH (1988) Carbon isotopes in photosynthesis, fractionation techniques may reveal new aspects of carbon dynamics in plants. Bioscience 38:328–336
20. O'Leary MH (1993) Biochemical basis of carbon isotope fractionation. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant water-relations. Academic Press, San Diego, pp 19–28
21. Alasalvar C, Shahidi F, Ohshima T, Wanasundara U, Yurttas HC, Liyanapathirana CM, Rodrigues FB (2003) Turkish tombul hazelnut (*Corylus avellana* L.). 2. Lipid characteristics and oxidative stability. J Agric Food Chem 51:3797–3805