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^{1}H NMR combined with chemometrics for the rapid detection of adulteration in camellia oils

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ABSTRACT

Proton nuclear magnetic resonance (^{1}H NMR) and chemometrics were employed to detect the adulteration of camellia oil (CAO) with 3 different cheap vegetable oils. With the intensity of 15 selected ^{1}H NMR signals as input variables, principal component analysis (PCA) showed good group clustering results for pure and nonpure CAO, but unsatisfied identification accuracy for the adulterated oil types, indicating relatively small difference among those oils. Whereas these difference could be revealed by orthogonal projection to latent structures discriminant analysis (OPLS-DA), with identification accuracy higher than 90%. Partial least squares (PLS) was further applied for the prediction of adulteration level in CAO. With less than 6 variables screened out by variable importance in the projection (VIP) scores as potential key markers, the developed PLS models showed better accuracy. The prediction results for 10 hold-out samples also confirmed that this method was accurate and fast for the detection of CAO adulteration.

1. Introduction

The genus Camellia originates from East Asia with many different breeds, and as one of the main species, the woody evergreen specie of Camellia oleifera is mainly produced in the southern provinces of China (Yang, Liu, Chen, Lin, & Wang, 2016). As one of popular edible vegetable oils, camellia oil (CAO) has become a significant ingredient in our daily diet due to its distinctive flavor and taste, high nutritional value, medical function and better storage stability than other edible oils (Haiyan, Bedgood, Bishop, Prenzler, & Robards, 2006). CAO has a large content of unsaturated fatty acids including palmitoleic acid (C16:1), oleic acid (C18:1, OA), linoleic acid (C18:2, LA), linolenic acid (C18:3), eicosenoic acid (C20:1) and docosenoic acid (C22:1) (Li, Kong, Shi, & Shen, 2016). Especially the oleic acid typically accounts for 74–87%, followed by linoleic acid ranging from 7 to 14% (Li et al., 2012). Its fatty acid composition is similar to that of olive oil, thus it is often honored as "Eastern Olive Oil". In addition to unsaturated fatty acids, camellia oil is also rich in Vitamins E, squalene and tea polyphenol (He, Zhou, Zhang, & Liu, 2011). Since it contains such a lot of natural antioxidants with various beneficial biological activities, CAO is useful in reducing the risk of high blood pressure, coronary heart disease, atherosclerosis, blood cholesterol, and regulating the nervous system, strengthening the immune system as well as preventing other diseases (Zeb, 2012). All those distinct properties make the CAO being very popular to consumers and sold at much higher price than other vegetable oils. In order to seek high profit, adulteration of CAO with other cheap vegetable oils was always found in the market. Due to being apparently similar to commercially qualified CAO, corn oil (CO), sunflower oil (SO) and rapeseed oil (RO) were most likely added into it by the unscrupulous traders.

Several conventional techniques have been reported to identify the adulteration of camellia oil, such as gas chromatography (GC) (Li, Huang, et al., 2016) and gas chromatography–mass spectrometry (GC–MS) (Xie, Liu, Yu, Song, & Hu, 2013). However, these methods are tedious, destructive, and often require complicated sample pretreatments. Thus, aiming to overcome these shortcomings, a lot of rapid, non-invasive and reproducible techniques have been applied recently including near infrared fourier transform raman spectroscopy (Weng, Weng, & Chen, 2006), total reflectance infrared spectroscopy (MIR-ATR) and fiber optic diffuse reflectance near infrared spectroscopy (FODR-NIR) (Wang, Lee, Wang, & He, 2006), electronic nose (Hai & Wang, 2006), ion mobility spectrometry (IMS) fingerprints (Liu et al., 2017), and differential scanning calorimetry (DSC) analysis (Li, Huang, et al., 2016). Compared with above mentioned methods, nuclear magnetic resonance (NMR) shows a lot of superiorities such as extreme speed, easy automation, remarkable selectivity, excellent

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repeatability, and capable of giving a complete view of chemical compositions with qualitative and quantitative information (Agiomyrgianaki, Petrakis, & Dais, 2010; Monteiro et al., 2009). In combination with the use of chemometric methods, NMR has become a preferred technique for the assessment of food authenticity, such as saffron (Petrakis, Cagliani, Tarantilis, Polissiou, & Consonni, 2017), roasted coffee (de Moura Ribeiro, Boralle, Redigolo Pezza, Pezza, & Toci, 2017), edible oils (Zhu, Wang, & Chen, 2017), honey (Siddiqui, Musharraf, Choudhary, & Rahman, 2017), milk (Santos, Pereira-Filho, & Colnago, 2016). Besides, ^{1}H NMR combined with principal component analysis (PCA) and partial least squares (PLS) were employed to determine the physicochemical properties of Brazilian crude oil (Duarte et al., 2016). ^{1}H NMR and orthogonal projections to latent structure-discriminant analysis (OPLS-DA) were applied for the discrimination of Korean and Chinese herbal medicines (Kang et al., 2008).

However, there are few researches reporting about the detection of adulterated CAO by applying ^{1}H NMR combined with PCA, OPLS-DA and PLS models. The objective of this research is to detect the adulteration of CAO with three cheaper vegetable oils including CO, SO and RO by combining application of ^{1}H NMR and chemometrics. In this research, 120 oil samples including 21 pure CAO, 89 nonpure CAO adulterated with other different types of oil at varied adulteration levels, and 10 hold-out samples were prepared and submitted to ^{1}H NMR experiment. PCA analysis was performed on their ^{1}H NMR data to explore the best bucket width, then on this basis, OPLS-DA and PLS were further employed to detect the adulteration and predict its adulteration levels as well.

2. Materials and methods

2.1. Samples

Four types of commercially available refined vegetable oils including CAO (n = 21, produced in different areas of Jiangxi Province), CO (n = 3, from different brands: Fulinmen, Jinlongyu and Changshouhua), SO (n = 3, from different brands: Fulinmen, Jinlongyu and Changshouhua), RO (n = 2, from different brands: Jinlongyu and Changshouhua), were provided by Jiangxi Province Bureau of Quality and Technical Supervision, and 10 samples for the out of sample validation were purchased from a local supermarket in Nanchang, China. All those samples were produced during January 2016 to May 2016, and were stored at 4 °C between delivery and NMR measurements to avoid compositional changes.

Eighty-one binary blend oil samples were prepared by adding either CO, SO or RO into CAO at percentages ranging from 5% to 80% (5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, v/v). A total of 120 samples were obtained and their NMR spectra were recorded immediately after preparation.

2.2. ^{1}H NMR spectroscopy

For each NMR sample preparation, 200 μL of the pure or blend oil sample was dissolved in 800 μL of deuterated chloroform ($CDCl_3$ 99.8%-d) containing tetramethylsilane (TMS 0.3% v/v). Next, 600 μL of the mixture was transferred into a standard 5 mm NMR tube for direct measurement. The reagent was purchased from Aladdin (Shanghai, China).

All one-dimensional ^{1}H NMR spectra were recorded at 600.38 MHz and 298 K on a Bruker AV 600 spectrometer (Bruker Corporation, Switzerland) equipped with a cryoprobe and a z-gradient. The acquisition parameters were as follows: time domain 32 K, 90° pulse width of 6.5 μs, spectral width of 13 ppm, acquisition time of 3 s and relaxation delay of 1 s; 32 scans and 4 dummy scans were accumulated for each free induction decay.

The NMR raw data sets were pre-processed in the MestReNova software (Mestrelab Reserch, Santiago de Compostela, Spain). Chemical shifts were calibrated by setting the peak of TMS as internal reference at 0.00 ppm to obtain good peak alignment. For ensuring a better quantitative evaluation of the signals, phase correction and baseline correction were performed automatically.

Table 1
Assignment of the signals of camellia oil ^{1}H NMR spectrum.

Signal	Chemical shift (ppm)	Multiplicity	Compound
1	5.42–5.29	m	−CH=CH− (all unsaturated fatty acids)
2	5.29–5.22	m	> CHOCOR (triacylglycerols)
3	4.36–4.24	dd	$-CH_2OCOR$ (triacylglycerols)
4	4.20–4.05		
	4.20–4.10	dd	$-CH_2OCOR$ (triacylglycerols)
	4.10–4.05	m	sn-1,3-diacylglycerols
5	4.04–3.98	m	sn-1,3-diacylglycerols
6	3.76–3.68	d	sn-1,2-diacylglycerols
7	2.84–2.79	t	$=CH-CH_2-CH=$(linolenyl group)
8	2.79–2.70	t	$=CH-CH_2-CH=$ (linoleyl group)
9	2.40–2.20	dt	$-OCO-CH_2-$ (all acyl groups)
10	2.08–1.94	m	$-CH_2-CH=CH-$ (oleyl, linoleyl and linolenyl groups)
11	1.70–1.50		
	1.70–1.67	s	Squalene
	1.67–1.50	m	$-OCO-CH_2-CH_2-$ (all acyl groups)
12	1.40–1.14	m	$-(CH_2)_n-$ (all acyl groups)
13	1.02–0.92	t	$-CH=CH-CH_2-CH_3$ (linolenyl group)
14	0.92–0.80	t	$-CH_2-CH_2-CH_2-CH_3$ (all acyl groups except linolenyl)
15	0.72–0.66		
	0.70	s	Stigmasterol
	0.68	s	β-Sitosterol
16	0.60–0.50	d	Triterpene alcohol (Cycloartenol)

s: single; d: doublet; t: triplet; m: multiplet; dt: double of triplet; dd: doublet of doublet.

In order to perform the statistical analysis, the spectra were integrated at four different types of bucket width over the region from 10 to 0.5 ppm excluding residual solvent signal (7.60–6.90 ppm) in the MestReNova (Piccinonna et al., 2016). On one hand, the intensity of 15 selected signals in Table 1 (except signal 9 that worked as a reference) were compared with the intensity of the signal of α-methylene protons of all acyl chains (signal 9, 2.40–2.20 ppm) that was normalized to 1000 (Mannina, Marini, Gobbino, Sobolev, & Capitani, 2010). For each resonance, this normalizing procedure gives an index, which is proportional to the molar ratio between each compound and the total amount of the fatty chains. Therefore, 15 buckets were obtained. On the other hand, the spectra were integrated at equal width of 0.26 ppm, 0.04 ppm and 0.01 ppm respectively, then normalised to total sum, obtaining 34 buckets, 220 buckets and 879 buckets. The constant bucketing width of 0.01 ppm and 0.04 ppm are commonly used in NMR metabolomics, while 0.26 ppm was as well employed since it coincided with the largest peak range (1.40–1.14 ppm) found over all selected signals in Table 1.

2.3. Fatty acid composition analysis of 10 hold-out samples

Fatty acid composition analysis was carried out using a 6890N gas chromatograph equipped with a flame ionization detector and a capillary column CP-Sil 88 (100 m × 0.25 mm × 0.39 mm, 0.20 μm). The analysis procedure was performed according to the method reported in our previous research (Yang, Chen, Zhang, Nie, & Xie, 2012).

2.4. Statistical analyses

Multivariate analysis of the acquired data was carried out by PCA, OPLS-DA and PLS in SIMCA P+ version 12 software (Umetrics, Sweden).