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Qualitative and quantitative analysis of β-sitosterol marker in virgin camellia oil and virgin olive oil

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Abstract

Camellia oil and olive oil with superior economic value are easily adulterated with other, cheaper oils. It is difficult to identify both oils by traditional methods because of their similar fatty acid profiles. In the present study, a novel method for qualitative and quantitative analysis of β-sitosterol using GC/MS and GC/FID was developed to identify camellia oil and olive oil. The method validation of β-sitosterol analysis showed good linearity and satisfactory values for recovery, accuracy, precision, and repeatability. The linear regression coefficient (R^2) of the calibration curve was 0.9985. An acceptable limit of detection (0.36 mg/100 g) and limit of quantification (1.20 mg/100 g) were achieved. The spiked recoveries were 95.0% to 100.3%. The relative standard deviation (RSD) of within-day precision was less than 3.26%, and the RSD of retention times and peak areas for repeatability were within 0.03% and 1.08%, respectively. The contents of β-sitosterol in virgin camellia oil and virgin olive oil were in the range of 14.1–30.2 mg/100 g and 94.3–173.2 mg/100 g, respectively, indicating that the β-sitosterol content in the former is seven times that in the latter, and β-sitosterol could be a potential marker for the authentication and adulteration detection of both oils.

Keywords: β-Sitosterol; virgin camellia oil (VCO); virgin olive oil (VOO); qualitative analysis; quantitative analysis.

Introduction

Camellia oil is the vegetable oil extracted from the seeds of *Camellia oleifera* Abel (Theaceae family), as one of the four top woody oil trees, widely cultivated in southern China, mainly including 14 Provinces/Autonomous Regions, such as Guangxi, Zhejiang, Jiangxi, Hunan, Anhui, and Fujian (Xie *et al.*, 2013; Wu *et al.*, 2020). It is estimated that more than 3.8 million ha are planted with *Camellia* species for oil in China and that most of this is in hilly or mountainous terrain, and the production of camellia oil in China is more than 600 000 t per year, which accounts for approximately 90% of global gross output (Shi *et al.*, 2020). Most camellia oil is refined in the same way as other commodity seed oils, and its sensory characteristics are much less intense and diverse than those of typical unrefined (virgin) food oils such as extra virgin olive oil (Long *et al.*, 2008). Owing to the extremely similar fatty acid (FA) profiles and physicochemical properties with olive oil, camellia oil is acclaimed as the 'oriental olive oil' (Zhou *et al.*, 2017). In addition, camellia oil has abundant unsaponifiable substances, such as phytosterols, squalene, α-tocopherol and phenolic compounds, which contribute to its nutritional benefits, desirable taste, and higher price (Shen *et al.*, 2021; Zheng *et al.*, 2021). Our team's previous research found that camellia oil and olive oil exerted similar effects on body weight and a majority of cardiometabolic profiles in middle-aged and elderly women at high cardiovascular risk, as these two oils were rich in monounsaturated FAs (MUFAs) (Wu *et al.*, 2022), and the findings also suggested that these two MUFA-rich oils are more beneficial for cardiometabolic profiles than soybean oil considering traditional Chinese eating habits. Because of the superior economic value of camellia oil and olive oil, they are easily adulterated with refined camellia oil, refined olive oil and other, cheaper vegetable oils (Meenu *et al.*, 2019; Kakouri *et al.*, 2021).

One of the major authenticity issues for the edible oil industry is the alteration of food ingredients labeling, whereby low-value oils are utilized for the illegal substitution of expensive ones without declaring it on the label. To protect the public's health and safeguard the fairness and impartiality of trade, camellia oil authenticity is attracting much attention from consumers, distributors, and producers. It is necessary to identify the authenticity of camellia oil to ensure its high intrinsic quality and ensure the healthy development of the oil industry (Shi *et al.*, 2020). In order to confirm the authenticity and detect adulteration of vegetable oil products, various specific markers have recently been proposed, such as FAs, triglycerides, phytosterols, squalene, and tocopherols (Shi *et al.*, 2022). Simultaneously, chromatographic techniques, as a

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result of their reliability and wide acceptability, were adopted for the qualitative and quantitative analysis of these markers (Dorota *et al.*, 2021; Rui *et al.*, 2021). The determination of the phytosterol composition is one of the most sensitive methods for the authentication and quality control of different vegetable oils (Deme *et al.*, 2021).

Phytosterols, like FAs, are the main components in vegetative oils, becoming important items in the Codex Alimentarius (Wang *et al.*, 2017). In vegetative oils, phytosterols are one of the significant minor components and can be found as free sterols or as conjugates of fatty acid steryl esters (Moreau *et al.*, 2018). As reported in the previous literature, more than 250 kinds of phytosterols have been detected, while the principal phytosterols present in vegetable oil are *β*-sitosterol, campesterol, brassicasterol, and stigmasterol (Singh, 2013; Lin *et al.*, 2018; Silva Almeida *et al.*, 2020). Therefore, *β*-sitosterol could be cognized as a suitable marker for the characterization and authentication of vegetable oils, as well as the detection of adulteration (Lukic *et al.*, 2013). Kamm *et al.* (2002) used *β*-sitosterol as a marker for the detection of vegetable oils in milk fat. The results showed that the screening and quantification of *β*-sitosterol allowed the detection of milk fat adulterations. As a consequence, quantifying *β*-sitosterol in vegetable oils has been of great interest to researchers. It has been reported in many studies that *β*-sitosterol, as the richest 4-desmethylsterol, was observed in many vegetative oil samples, ranging from 339 to 5183 mg/kg, such as in rice oil (5183 mg/kg), corn oil (3580 mg/kg), rice bran oil (3343 mg/kg), rapeseed oil (3036 mg/kg), sesame oil (2796 mg/kg), soybean oil (1088 mg/kg), and palm superolein (559 mg/kg) (Kolenc *et al.*, 2020; Shi *et al.*, 2021).

Several studies have been conducted for the detection of camellia oil and olive oil adulteration by quantifying phytosterols (Kolenc *et al.*, 2020; Shi *et al.*, 2021). However, few studies have been conducted proposing quantifying a specific kind of phytosterol, for instance, *β*-sitosterol, for the authenticity identification of these two edible oils. Furthermore, the method validation of quantifying *β*-sitosterol for the detection of adulteration has rarely been studied systematically. Therefore, as a marker for detection and distinction of camellia oil and olive oil, a reliable method to analyze *β*-sitosterol qualitatively and quantitatively is needed.

This study aimed to develop a determination method for the detection and distinction of camellia oil and olive oil. A simplified approach was proposed for the extraction of *β*-sitosterol in virgin camellia oil (VCO) and virgin olive oil (VOO), and the extractive matrix was detected without derivatization. Qualitative and quantitative analyses of *β*-sitosterol were performed using gas chromatography/mass spectrometry (GC/MS) and gas chromatography/flame ionization detector (GC/FID). Method validation was investigated based on the values of linearity, recovery, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and repeatability. Furthermore, *β*-sitosterol in VCO and VOO were compared for further study.

Materials and Methods

Materials

Camellia seeds (cultivars named Changlin3, Changlin4, Changlin8, Changlin21, Changlin23, Changlin27, Changlin40, Changlin53, Changlin55, Changlin166, and Changlin180) and olive fruits (cultivars named Pixiaoli, Zhongshan, Ezhi, and Laixing) were provided by the Institute of Subtropical Forestry, Chinese Academy of Forestry.

FA methyl ester qualitative mixture was purchased from NU-CHEK Corporation (Elysian, MN, USA). *β*-Sitosterol standard was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Distilled water was used throughout the experiments. All other reagents were of analytical or HPLC grade.

Preparation of VCO and VOO

VCO extraction was performed as described previously with some modifications (Liu *et al.*, 2021). Briefly, the camellia seeds were dried in an oven at 50 °C, followed by pulverizing. The mixture of camellia seed powder and water (1:5, mass concentration) was shaken at 160 r/min for 30 min in a thermostat water bath (50 °C) coupled with ultrasound. Then, the substrate was centrifuged for the separation and extraction of the oil. The 11 VCO samples were stored in the dark at 4 °C, which were denoted as VCO-3, VCO-4, VCO-8, VCO-21, VCO-23, VCO-27, VCO-40, VCO-53, VCO-55, VCO-166, and VCO-180. The VOO samples were manufactured by mechanical means directly from the four olive fruits, as the cold-pressed method performed as described by Jukic Spika *et al.* (2021), and were stored in the dark at 4 °C, which were denoted as VOO-P, VOO-Z, VOO-E, and VOO-L.

Analysis of FAs in VCO and VOO by GC

FAs in oil were converted into the corresponding FA methyl esters (FAMEs) by KOH-BF_3 solution (Shen *et al.*, 2010), and then analyzed according to the methods of Li *et al.* (2021). Gas chromatograph (GC) 7890A (Agilent, Santa Clara, CA, USA) was coupled with a flame ionization detector (FID) and a DB-23 capillary column (60 m× 0.25 mm×0.25 μm; Agilent). The injection volume was 1 μL with a split ratio of 1:200. The injector temperature was 250 °C and the detector temperature was 270 °C. Programmed temperature mode: initiating at 80 °C for 2 min, heating up to 140 °C at 10 °C/min (hold for 2 min), and to 180 °C at 10 °C/min (hold for 6 min), and then to 220 °C at 5 °C/min (hold for 8 min), and to 230 °Cat 10 °C/min (hold for 3 min). Data acquisition and processing were performed with Agilent ChemStation.

β-Sitosterol extraction

The pretreatment of VCO and VOO samples for *β*-sitosterol extraction was carried out based on the preliminary research in our laboratory. One gram of camellia oil was saponified with 5 mL of 2 mol/L KOH-CH_3CH_2OH solution at 85 °C for 1 h. The matrix was cooled to room temperature, and then 5 mL of saturated sodium chloride solution and 4 mL of *n*-hexane were added twice to extract unsaponifiable matter. Both combined supernatants were collected after phase separation, and washed with 4 mL of distilled water. Then, the upper fractions were separated into a tube by adding anhydrous Na_2SO_4 and centrifuging at 2000 r/min for 5 min. The supernate was then collected and blown with nitrogen to dryness. Finally, 2 mL of *n*-hexane was added and the sample solutions were filtered through a 0.45-μm nylon syringe filter for further analysis of *β*-sitosterol.

Analysis of β-sitosterol through GC/MS and GC/FID

For qualitative analysis of β-sitosterol, the β-sitosterol samples were analyzed by an Agilent 7890A GC equipped with an Agilent 6890B mass detector (Mahboob *et al.*, 2022). AHP-5MS capillary column (30 m×0.25 mm×0.25 μm; Agilent) was used to separate the analytes. The flow rate of helium as the carrier gas was 0.7 mL/min. The samples were injected at 250 °C with a volume of 1 μL (split ratio 50:1). The column temperature was initiated at 100 °C (hold for 2 min), and increased to 290 °C at 15 °C/min (hold for 10 min). The source temperature was set at 230 °C, and the electron impact energy was 70 eV. The temperature of the transfer line was 250 °C, and the solvent delay was 3.5 min for each run. The values of the ions were selected as *m/z* 231.2 and 329.3, and the retention times (RTs) and mass spectra (MS) data were analyzed with available standards.

For quantitative analysis, β-sitosterol was analyzed by GC/FID. The chromatographic conditions were as follows: HP-5MS capillary column (30 m×0.25 mm×0.25 μm; Agilent); split injector with a volume of 1 μL (split ratio 50:1); the temperature of the injector was 250 °C; the column temperature was initiated at 100 °C (hold for 2 min) and increased to 290 °C at 15 °C/min (hold for 10 min). The temperature of FID was set at 270 °C.

Method validation

The method validation was assessed with the following criteria: linearity, recovery, accuracy, precision, LOD, LOQ, and repeatability. The linearity of the analytical curve was analyzed with 8 points in the concentration range from 10 to 600 μg/mL (10, 50, 100, 200, 300, 400, 500, and 600 μg/mL), and the regression coefficient was calculated. The recovery of β-sitosterol was determined according to the standard addition method by spiking the VCO samples at two levels (10 mg/100 g and 40 mg/100 g) with three replicates. The accuracy was evaluated by comparing the calculated concentration of spiked samples with the actually added concentration and expressed as the spiked recovery. The precision (within-day) was evaluated by the relative standard deviation (RSD) values of the measured concentrations on the same day. The LOD and LOQ for β-sitosterol were determined by using spiked blank VCO samples, and they were calculated from the ratio of the signal produced by the minimum concentrations of β-sitosterol to the noise signal, that is, the signal-to-noise (S/N) ratio. S/N ratios of 3 and 10 were found for LOD and LOQ, respectively (Pokkanta *et al.*, 2019). For repeatability (inter-laboratory precision), the RSD of RTs and peak areas (PAs) were calculated by five replicates.

Statistical analysis

Data were expressed as mean±standard deviation. Statistical analysis was conducted by ANOVAs with SPSS Statistics (version 20.0; IBM, Armonk, NY, USA). Differences were statistically significant at $P<0.05$.

Results and Discussion

FA composition of VCO and VOO

Typical GC chromatograms for separation of FAs of VCO and VOO are shown in Figure 1 and the FA composition is shown in Table 1. From the data, we can see that the major FAs of VCO were oleic acid (C18:1n-9, 76.1%–82.0%), followed by palmitic acid (C16:0, 9.0%–10.8%), linoleic acid (C18:2n-6, 7.1%–12.6%), and stearic acid (C18:0, 1.3%–2.0%). This

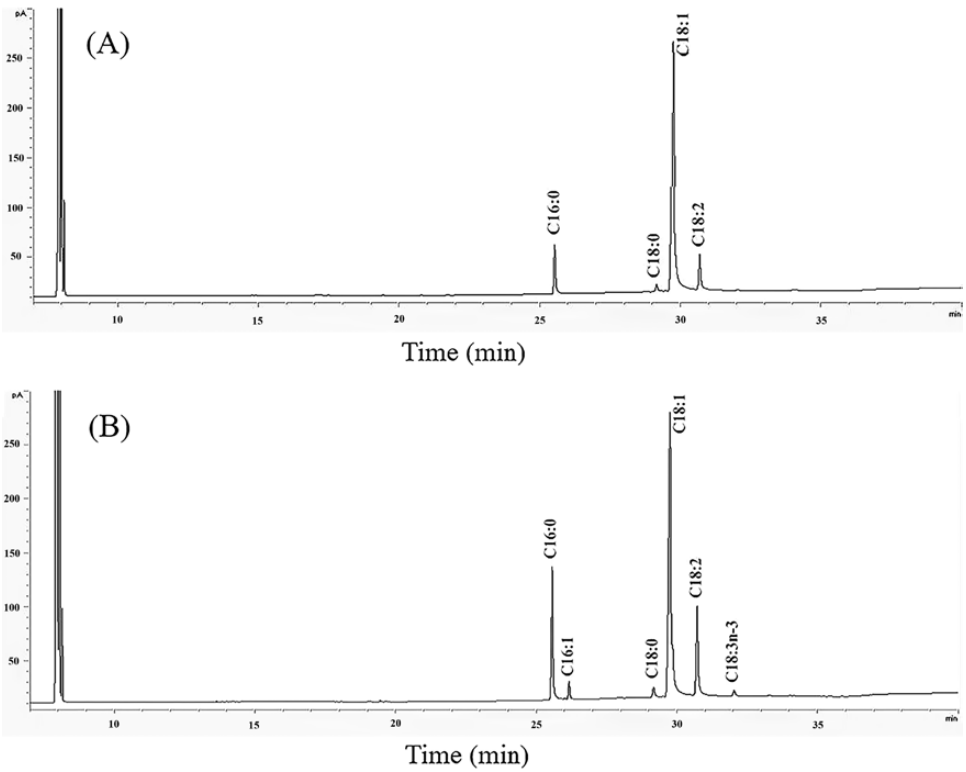

Figure 1. Typical GC chromatograms for separation of FAs of VCO (A) and VOO (B).

Table 1. FA composition of VCO and VOO

Cultivars	Palmitic acid (C16:0) (%)	Palmitoleic acid (C16:1) (%)	Stearic acid (C18:0) (%)	Oleic acid (C18:1n-9) (%)	Linoleic acid (C18:2n-6) (%)	α-Linolenic acid (C18:3n-3) (%)
VCO-3	9.5±0.2^{e}	-	1.7±0.2^{bc}	76.1±0.4^{bc}	12.6±0.1^{b}	–
VCO-4	9.0±0.1^{f}	-	1.4±0.1^{e}	80.3±0.3^{a}	9.4±0.1^{d}	–
VCO-18	9.3±0.1^{e}	-	1.9±0.1^{a}	79.1±0.2^{a}	9.7±0.1^{d}	–
VCO-21	10.5±0.2^{d}	-	1.8±0.2^{b}	78.2±0.3^{ab}	9.5±0.3^{d}	–
VCO-23	9.9±0.1^{de}	-	1.6±0.2^{c}	80.0±0.5^{a}	8.5±0.4^{de}	–
VCO-27	10.8±0.3^{d}	-	1.7±0.1^{c}	77.3±0.3^{b}	10.2±0.2^{c}	–
VCO-40	10.1±0.2 ^{d}	-	2.0±0.1^{a}	79.2±0.3^{a}	8.6±0.4^{de}	–
VCO-53	9.4±0.2^{e}	-	1.3±0.1^{f}	79.7±0.6^{a}	9.5±0.4^{d}	–
VCO-55	9.7±0.1^{e}	-	1.9±0.2^{a}	79.6±0.0^{a}	8.8±0.3^{de}	–
VCO-166	10.1±0.1^{d}	-	1.6±0.1^{cd}	78.2±0.2^{b}	10.2±0.1^{c}	–
VCO-180	10.6±0.1^{d}	-	1.4±0.1^{e}	78.4±0.2^{a}	9.6±0.0^{d}	–
VOO-P	15.5±0.2^{b}	1.6±0.1^{c}	1.8 ±0.1 ^{b}	77.0±0.4^{b}	3.3±0.1^{h}	0.8±0.1^{c}
VOO-Z	18.0±0.2^{a}	2.1±0.1^{b}	1.6±0.1^{d}	62.6±0.4^{d}	14.9±0.2^{a}	0.9±0.1^{b}
VOO-E	14.7±0.4^{c}	3.8±0.1^{a}	1.0±0.1^{g}	74.0±0.4^{c}	5.3±0.1^{g}	1.1±0.1^{a}
VOO-L	17.3±0.3^{a}	1.6±0.0^{c}	1.3±0.1^{f}	75.0±0.2^{c}	4.1±0.1^{f}	0.7±0.0^{c}

All data are presented as mean±standard deviation.
In each column, different letters represent significant differences (p<0.05).

was consistent with the research by Wei *et al.* (2016), who found that camellia oil was rich in MUFA (mainly oleic acid), which accounted for approximately 78.3% of eight commercial camellia oil samples from southern China. The major FAs of VOO were oleic acid (C18:1n-9, 62.6%–78.9%), followed by palmitic acid (C16:0, 10.4%–18.0%), linoleic acid (C18:2n-6, 3.3%–14.9%), stearic acid (C18:0, 1.0%–2.9%), palmitoleic acid (C16:1, 0.6%–3.8%), and α-linolenic acid (C18:3n-3, 0.6%–2.4%). In addition, it is apparent from Table 1 that the main difference in FA composition between different cultivars of camellia oil from the same place of origin was for oleic acid (C18:1n-9), linoleic acid (C18:2n-6) and palmitic acid (C16:0). The same result was found in olive oil. This was in agreement with the research by Su *et al.* (2014), who studied the chemical composition of seed oils in different native *Camellia* species in Chinese Taiwan. Liu *et al.* (2021) also analyzed the FA composition between eight different cultivars of camellia oil from the same place, as well as other two cultivars of camellia oil from another place, and similar results were obtained. A large number of previous studies proved that the Mediterranean diet based on olive oil is associated with a decreased incidence of cardiovascular disease (Estruch *et al.*, 2018; George *et al.*, 2019). Camellia oil, with extremely similar FA profiles to olive oil, would be a great potential edible oil product because of the rise of China's economy.

Figure 2 demonstrates the comparison of FA composition of VCO and VOO, and it is obvious that both VCO and VOO were dominated by oleic acid, followed by palmitic acid, linoleic acid, and stearic acid as the major FAs. This was in agreement with the results revealed by Shen *et al.* (2021) that camellia oil and olive oil were very similar in their FA composition and were rich in oleic acid. The high oleic acid in VCO and VOO indicated promising applications for health promotion, which includes lowering cholesterol and triglycerides in the blood serum samples (Tutunchi *et al.*, 2020; Sakurai *et al.*, 2021). However, low contents of palmitoleic acid and α-linolenic acid were detected only in VOO, which was also demonstrated by the previous discoveries of Liu *et al.* (2021), Christopoulou *et al.* (2004), and Martinez *et al.* (2014). These results indicated the potential application of FA composition for the rough classification of VCO and VOO. For better discrimination, other indicators such as β-sitosterol could be taken into consideration for further identification of VCO and VOO (Shi *et al.*, 2020).

Qualitative analysis of β-sitosterol with GC/MS

The identification of β-sitosterol was confirmed by GC/MS, on the basis of investigations of non-derived unsaponifiable

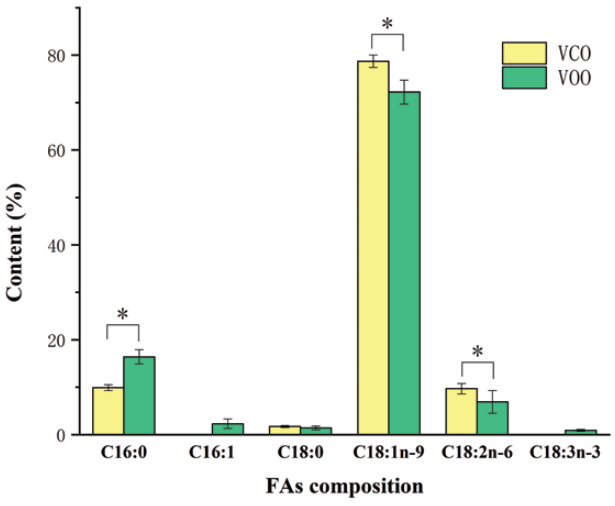

Figure 2. Comparison on FAs composition of VCO and VOO. *Significant difference (P<0.05).

extracts of VCO and VOO. As good separation and resolution of the *β*-sitosterol peak (RT=19.9 min) were reflected in the GC/MS chromatograms, it was convenient to identify the compound by comparing its RT with that of the standard solution. Additionally, the matching of mass spectra of the compound in oil samples and standard solution with library data further confirmed of *β*-sitosterol (Figure 3), and the chemical structures of the identified compounds were identified. Thus, the qualitative analysis of *β*-sitosterol by GC/MS was successful. These results verified that the pretreatment process was effective for the qualitative analysis of *β*-sitosterol; similar results were reported by Silva Almeida *et al.* (2020), who optimized a one-step rapid extraction of phytosterols by means of response surface methodology and proved that the new analytical method is precise and accurate.

Sample preparation is one of the key and critical steps for the determination of *β*-sitosterol in oil to remove interferences and pre-concentrate the analytes (Kardani *et al.*, 2011). In the traditional method, preparation for the analysis of *β*-sitosterol should be carried out by steps that include (i) saponification with or without acid hydrolysis, (ii) unsaponifiable matter extraction, (iii) purification by solid-phase extraction (SPE) or thin-layer chromatography (TLC), and (iv) derivatization with trimethylsilyl for fragmentation ion studies (Zhang *et al.*, 2005; Gachumi and El-Aneed, 2017; Secmeler and Ustundag, 2017; Vu *et al.*, 2019). However, in this study, the extraction of *β*-sitosterol was performed without the use of SPE cartridge or TLC, and the determination was performed without derivatization, which avoids excessive manipulation of the samples and shows the potential to be applied in future research.

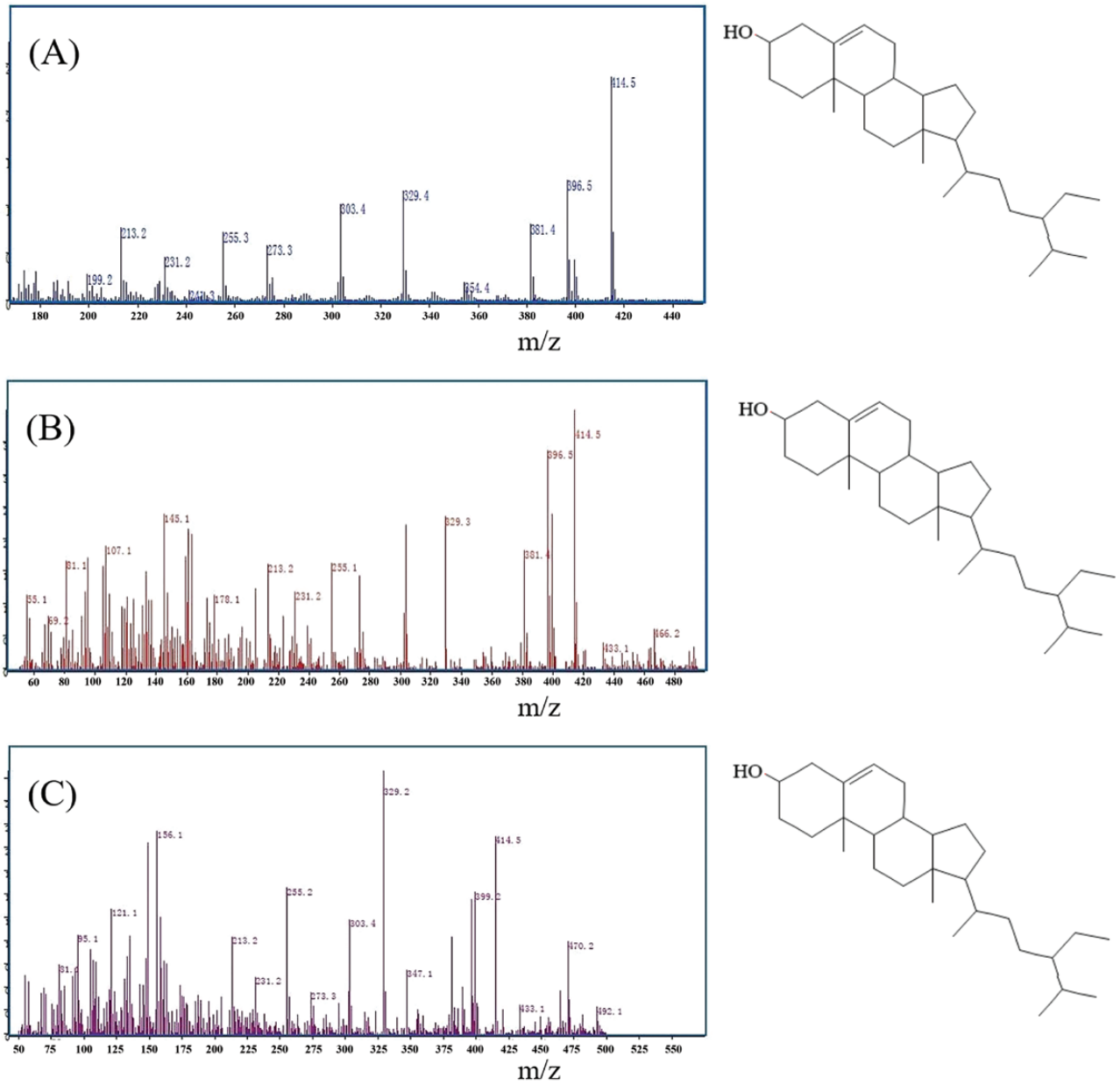

Figure 3. MS spectra and structural formula of the β-sitosterol standard sample (A), β-sitosterol in VCO (B), and β-sitosterol in VOO (C).

Quantitative analysis of β-sitosterol with GC/FID and method validation

For quantitative analysis of β-sitosterol, GC/FID has been considered a reliable approach for a long time (Srigley and Haile, 2015; Alvarez-Sala *et al.*, 2016; Duong *et al.*, 2016). To evaluate the method performance, accuracy studies were performed as described in previous studies (Noyon *et al.*, 2016). Linearity of the presented method was assessed by regression analysis of the calibration curve. The correlation coefficient (R^2) was 0.9985 (Table 2), indicating good linearity could be obtained in the working range of 10–600 μg/mL (Cecchi *et al.*, 2017). Furthermore, the LOD and LOQ values were 0.36 mg/100 g and 1.20 mg/100 g, respectively. The oil samples were spiked with low and high concentrations of β-sitosterol, and the recovery values varied from 95.0% to 100.3%, which indicated that the accuracy of this method was acceptable. The RSD of within-day precision in recovery tests was less than 3.26% (n=3). Five repeated experiments were applied to evaluate the repeatability (inter-laboratory precision) of the presented method, and the RSDs for RTs and PAs were less than 0.03% (n=5) and 1.08% (n=5), respectively.

The proposed quantitative method for the determination of β-sitosterol in this work was compared to the other published methods in Table 3. It is apparent that the proposed method provides outstanding analysis performance with low LOD and LOQ, which was an order of magnitude lower than the work of determination of β-sitosterol in soybean oil, corn oil, sunflower oil, canola oil, and olive oil (Silva Almeida *et al.*, 2020). This work also has the advantage of being faster, being 10–20 min faster than some published works (Kardani *et al.*, 2011; Secmeler and Ustundag, 2017). All method validation parameters suggested that the proposed method meets the authentication requirements and is a suitable quantification tool for the determination of β-sitosterol (Kim *et al.*, 2016).

β-Sitosterol in VCO and VOO

β-Sitosterol, as one of the major phytosterols with 29 carbon atoms (Garcia-Llatas *et al.*, 2021), is similar to cholesterol both in structure and function (Wang *et al.*, 2019; Fernandez-Cuesta *et al.*, 2012). The remarkable function of β-sitosterol is the lowering of low-density lipoprotein cholesterol levels as a useful dietary supplement (Jones and AbuMweis, 2009; Kmiecik *et al.*, 2011). In addition, β-sitosterol shows significant anti-inflammation, anti-ulceration, anti-bacterial, anti-fungus, and anti-tumor effects (Hidalgo *et al.*, 2009; Gylling *et al.*, 2014; Patel *et al.*, 2017; Shyamaladevi and Selvaraj, 2020). As investigated in several previous sudies, β-sitosterol was considered a valid marker for adulteration detection in several edible oils (Anupama *et al.*, 2016; Skiada *et al.*, 2020).

Table 2. Standard curve, correlation coefficient, within-day precision, recovery, LOD, LOQ, and quantitative analysis of β-sitosterol with GC-FID

Standard curve	Correlation coefficient (R^2)	Original sample (mg/100 g)	Spiked level (mg/100 g)	Within-day precision (RSD, %, n=3)	Recovery (%)	LOD (mg/100 g)	LOQ (mg/100 g)
y=0.3163x–1.7637	0.9998	20	10	3.26	100.3	0.36	1.20
			40	1.70	95.0		

Table 3. Comparison on the performance of the method in this work with published methods for β-sitosterol analysis

Sample	Quantitative analysis instrument	LOD (mg/100 g)	LOQ (mg/100 g)	Analysis time (min)	Reference
Virgin camellia oil Virgin olive oil	GC-FID	0.36	1.20	24.6	This work
Corn germ oil Corn oil Sesame oil Peanut oil Walnut oil	SPE-HPLC	0.60	2.00	25	Wang *et al.*, 2019
Soybean oil Corn oil Sunflower oil Canola oil Olive oil	GC-FID	2.00	6.50	25	Silva Almeida *et al.*, 2020
Fourteen rice bran Nine vegetable oil	HPLC-FLD	0.73	2.17	35	Pokkanta *et al.*, 2019
Olive oil	GC-FID	2.80	8.40	40	Secmeler and Ustundag, 2017
Sunflower oil Soybean oil	GC-FID	0.70	2.40	30	Kardani *et al.*, 2011

With the method developed in this study, β-sitosterol in 11 VCO samples and 4 VOO samples was determined. The contents of β-sitosterol in the VCO samples were diversified depending on the variety of camellia seeds cultivars, as well as in VOO. This may be due to genetic, cultivar, and agronomic factors (Shi *et al.*, 2019). The content of β-sitosterol in the VCO samples was in the range of 14.1–30.2 mg/100 g (with an average of 19.6 mg/100 g). However, the content of β-sitosterol in the VOO samples was in the range of 94.3–173.2 mg/100 g (average 145.4 mg/100 g), which was seven times that in VCO, suggesting that VOO is a good source of β-sitosterol. Consequently, the significant association between the content of β-sitosterol marker and type of oils could be established based on the single-factor ANOVA (Dou *et al.*, 2018). Taken together, these results suggested that β-sitosterol could be considered as a specific marker for the identification of VCO and VOO, as well as for the detection of VCO and VOO adulteration.

The contents of β-sitosterol were also compared with those reported in other studies. Silva Almeida *et al.* (2020) reported that the content of β-sitosterol in five commercial extra virgin olive oil was in the range of 121.9–159.9 mg/100 g, and Wang *et al.* (2017) reported that the content of β-sitosterol in eight commercial camellia oil was in the range of 12.3–29.4 mg/100 g. The results in our research were consistent with the findings reported in the above studies, which confirmed that the process of β-sitosterol extraction in this research was viable and efficient.

Conclusions

In this work, for the purpose of authentication of VCO and VOO, a reliable and effective method was established for qualitative and quantitative analysis of β-sitosterol. The extraction of β-sitosterol was performed without the use of SPE cartridge or TLC, and the determination was performed without derivatization, which avoids excessive manipulation of the samples. Method validation suggests that the improved method exhibited considerable advantages for its good linearity, recovery, accuracy, precision, and repeatability. Moreover, there was a significant difference in the content of β-sitosterol in VCO and VOO, which suggests that β-sitosterol would be a specific marker for future investigation of adulteration detection in VCO and VOO. In summary, our work provides a feasible approach for the determination of suitable characteristic marker in VCO and VOO, which provides promising applications in the authentication of other edible oils.

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Author Contributions

Yang Li: Conceptualization, methodology, data curation, writing original draft preparation, funding acquisition. Minyu Wu: Formal analysis, data curation. Liang Zhai: Methodology, investigation, Software. Hui Zhang: Formal analysis, data curation, resources. Lirong Shen: Project administration, supervision, conceptualization, review and editing, funding acquisition.

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Conflict of Interest

The authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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