



The use of Raman spectroscopy and chemometrics for the discrimination of lab-produced, commercial, and adulterated cold-pressed oils

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ABSTRACT

In this study, lab-produced cold-pressed oil samples of black cumin (CPBCO), almond (CPAO), and walnut (CPWO) were compared with their commercial substitutes. Additionally, adulteration of cold-pressed oils with corn and sunflower oils was determined. Chemical information and fatty acid composition of the samples were obtained using Raman spectroscopy and gas chromatography, respectively. Raman spectra were analyzed with principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). The developed PCA models for the discrimination of CPBCO, CPAO, and CPWO from the adulterated samples explained 93.35%, 96.24, and 94.25% of the cumulative variances, respectively. Root mean squared error of calibration (RMSEC), root mean squared error of cross-validation (RMSECV), and root mean squared error of cross-prediction (RMSEP) values were found to be between 0.06 and 0.21, 0.03–0.11, and 0.05–0.21; 0.08–0.24, 0.06–0.15, and 0.08–0.25; and 0.05–0.18, 0.03–0.10, and 0.09–0.20 for the PLS-DA models of CPBCO, CPAO, and CPWO adulterations. The developed PCA models for the discrimination of CPBCO, CPAO, and CPWO from commercial samples explained 99.64%, 91.27, and 92.11% of the cumulative variances, respectively. These results indicate that Raman spectroscopy coupled with chemometrics could be potentially useful both for the discrimination of cold-pressed oils and for the rapid determination of adulteration with cheaper oils.

1. Introduction

Edible oils are of great importance in human nutrition as a source of unsaturated fatty acids and other bioactive substances, and their nutritional quality is adversely affected by the production processes of oils. Among the edible oil production techniques, cold-pressing – which is introduced as a simple, cheap, and easy process – is a technique that has gained popularity in recent years. Cold-pressed oils obtained using this technique are also favored by consumers (Gao, Cao, Liu, Jin, & Wang, 2019). The fact that cold-pressing does not involve any heat treatment or chemical solvents to produce seed oil allows for obtaining a higher amount of aromatic oil rich in valuable bioactive substances (Lutterodt et al., 2010; Prescha, Grajzer, Dedyk, & Grajeta, 2014; Ramadan, Asker, & Tadros, 2012). On the other hand, the oil yield of the cold-pressing technique is lower than the solvent extraction, resulting in a significant increase in the cost of production (Aydeniz, Güneşer, & Yilmaz, 2014). Due to the high prices of cold-pressed oils, their availability is

limited, and thus they become an attractive target for fraudulent activities. Like other precious oils, cold-pressed oils may be diluted with any cheaper oils such as sunflower oil, or even produced completely from different raw materials and just aromatized to be accepted by consumers (Jee, 2002; Li, Wang, Zhao, Ouyang, & Wu, 2015).

Cold-pressed oil is a good source of bioactive components, and a great deal of cold-pressed oil is commercially produced and sold in Turkish food and the medical market. Due to the presence of health promoting bioactive compounds in the oils of black cumin (Ramadan et al., 2012), almond (Özcan, Al Juhaime, Ghafour, Babiker, & Özcan, 2020), and walnut (Gao et al., 2019), they are among the most heavily demanded cold-pressed oils by consumers. Black cumin oil, having antioxidant and antimicrobial activity, is one of the most important dietary supplement products (Ramadan et al., 2012). Not only its unsaturated fatty acid content demonstrating beneficial effects on lipoprotein profiles in the blood and its ability to lower the risk of cardiovascular diseases, but also its minor components including

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phytosterols, tocopherols, and squalene make almond oil a beneficial edible oil. Because of the large amounts of vitamins E and K, it is also an indispensable oil used in the cosmetic industry (Wang, Wang, Xiao, & Xu, 2019; Čolić, Zec, Natić, & Fotirić-Aksić, 2019). Besides the valuable unsaturated fatty acids it has, walnut oil also contains a vast amount of micronutrients such as tocopherols, polyphenols, and phytosterols exhibiting strong antioxidant properties (Gao et al., 2019).

The determination of counterfeit food remains a long-standing challenge for the researchers and control authorities. Therefore, novel analytical methods to be used for the control of food adulteration are of great importance (Deelstra, Burns, & Walker, 2014). Chromatographic methods offer promising results and are preferred as the official analysis methods in the quality control of vegetable oils (Aparicio & Aparicio-Ruiz, 2000). While gas chromatography combined with mass spectroscopy (GC/MS) has been used for the authentication of edible oils in several studies (Sun et al., 2015; Xing et al., 2019), it still has some disadvantages like the requirement of complex and time-consuming sample preparation steps involving toxic chemicals and the need for skilled personnel (Yazgan Karacaglar, Bulat, Boyaci, & Topcu, 2019). On the other hand, spectroscopic methods, namely fluorescence spectroscopy (Ge, Chen, Liu, & Zhao, 2014; Li et al., 2015; Temiz, Tamer, Berkkan, & Boyaci, 2017), mid-infrared spectroscopy (Wu, Li, & Tu, 2015; Zhao, Dong, Zheng, Jiao, & Lang, 2015), Fourier transform infrared spectroscopy (FT-IR) (Nurrulhidayah, Che Man, Al-Kahtani, & Rohman, 2011; Rohman & Ariani, 2013), and Raman spectroscopy (Velioglu et al., 2016) have been successfully used in the discrimination and authenticity determination of oils.

Raman spectroscopy is a non-invasive technique which gives detailed chemical information about the sample without any requirement of sample preparation step and allows the researchers to achieve promising results in a very short time (Beattie, Bell, & Moss, 2004). Raman spectroscopy has been successfully used to detect adulteration in cold-pressed oils. McDowell et al. employed Raman spectroscopy for the determination of adulteration in cold-pressed rapeseed oil (McDowell, Osorio, Elliott, & Koidis, 2018). This technique was also used by Yildiz Tiryaki and Ayvaz for the detection of adulteration in cold-pressed extra virgin olive oil (Yildiz Tiryaki & Ayvaz, 2017). On the other hand, adulteration in black cumin seed oil (Arslan et al., 2019), wheat germ oil (Arslan & Çağlar, 2019), grape seed oil (Karuk Elmas et al., 2019) and pomegranate seed oil (Uncu, Napiórkowska, Szajna, & Ozen, 2020) was determined using other spectroscopic techniques such as FT-IR, fluorescence, and UV-vis spectroscopy.

A closer look at the studies in the literature proposing different techniques for the determination of the adulteration of cold-pressed oils has demonstrated us that all of these studies utilized commercial oil samples (Arslan et al., 2019; Arslan & Çağlar, 2019; Karuk Elmas et al., 2019; McDowell et al., 2018; Rodríguez, Gagnet, Farroni, Percibaldi, & Buera, 2019; Uncu et al., 2020). Although the quality of the commercial samples used was reported to be guaranteed by the suppliers in many of these studies, it is obvious that the use of the lab-produced cold-pressed oil samples produced for the study would contribute to the originality of the authentication studies. Hence, considering the abovementioned contradiction in the literature, cold-pressed almond, walnut, and black cumin oils were produced under laboratory conditions and used as the lab-produced cold-pressed oil samples in the present study.

In this study, we investigated the potential of Raman spectroscopy coupled with PCA and PLS-DA to determine the adulteration of three different cold-pressed oils and to establish the discrimination between lab-produced and commercially provided cold-pressed oil samples. Black cumin (*Nigella sativa*), almond (*Prunus amygdalus*), and walnut (*Juglans regia* L.) oils, which are popular cold-pressed oils in the food and medical market due to their high nutritional quality and market value were used. Thirty-three samples of lab-produced cold-pressed oils were produced in the laboratory. Commercial corn and sunflower oils were used to prepare the synthetically adulterated oil samples of cold-pressed

almond, black cumin, and walnut oils. Additionally, twenty-four commercial oil samples, claimed to be produced by cold pressing, were collected from the Turkish food and medical market. Raman spectra of all samples were obtained and analyzed using two different chemometric methods, namely PCA and PLS-DA. As an unsupervised method, PCA was used to conduct an explorative analysis. PLS-DA is one of the most widely used supervised classification techniques, and it was used to build the classification models in the present study.

Detailed information about the fatty acid composition of the samples was also acquired through GC-FID (Gas Chromatography-Flame Ionization Detection) analysis.

2. Material and methods

2.1. Materials

Raw black cumin seed samples ($n = 12$, 200 g for each) were obtained from primary producers located in Burdur and Ankara, Turkey. Raw almond ($n = 12$, 200 g for each) and raw walnut ($n = 9$, 200 g for each) samples were obtained from primary producers located in Tekirdag, Turkey. These raw samples were used in cold-pressed oil production in the laboratory.

The commercial cold-pressed oil samples, including 8 black cumin, 11 almond and 5 walnut oils were obtained from food and medical markets in İzmir, Turkey. Based on what was stated on their labels, all samples were produced by cold pressing, and this information was verified by the producer and/or seller. The commercial samples were obtained in original glass bottles (differing from 20 to 50 mL) and stored in a dark place at room temperature until the analysis.

The cold-pressed oil samples produced in the laboratory were coded as cold-pressed black cumin oils (CPBCO1...CPBCO12), cold-pressed almond oils (CPAO1...CPAO12), and cold-pressed walnut oils (CPWO1...CPWO9). Commercial sunflower oil and corn oil samples were obtained from the local market and used for the preparation of synthetically adulterated samples. The commercial samples were coded as follows: black cumin oils (CBCO1...CBCO8), almond oils (CAO1...CAO11), and walnut oils (CWO1...CWO5). All chemicals used were of analytical grade.

Synthetically adulterated samples at levels of 25% and 50% were produced as follows: First of all, a master mix of cold-pressed oil was prepared using lab-produced cold-pressed oil samples. Then, sunflower oil and cold-pressed oil was mixed homogeneously with a magnetic stirrer at 25:75 (v/v), and 50:50 (v/v) levels, and the adulterated samples were coded as CPBCO(25 or 50)%SO, CPAO(25 or 50)%SO and CPWO(25 or 50)%SO for black cumin, almond, and walnut oils, respectively. The same procedure was employed for corn oil adulteration, and the adulterated samples were coded as CPBCO(25 or 50)%CO, CPAO(25 or 50)%CO, and CPWO(25 or 50)%CO. Table 1 summarizes the sample codes and experimental procedures followed in the present study.

2.2. Production of cold-pressed oil samples

Black cumin samples were used directly where the almonds were roughly grounded before the pressing. Oil samples were obtained with a cold-pressing method described by Kiralan, Özkan, Bayrak, and Ramadan (2014) with slight modifications. Raw materials were pressed at room temperature (25 °C) without any thermal treatment by using a laboratory-scale cold pressing machine (Dikmaksan, Antalya, Turkey) until the beginning of oil drainage. The oil was collected in a flask by using a cotton filter mounted on a glass funnel. Then, the oil sample was centrifuged in a refrigerated centrifuge (Universal 32 R, Hettich, Tuttingen, Germany) at 10,000 rpm (9391 g) for 10 min at room temperature. The clear oil phase was collected using a Pasteur pipette and put into an amber bottle with nitrogen gas headspace and stored at -18°C until analysis.

Table 1

The sample codes and experimental procedures followed in the study.

Sample	Type and number of the samples	Sample codes	Experiment	Data analyses
Commercial cold-pressed oils	Black cumin oil (n = 8)	CBCO1	GC	ANOVA
		CBCO8	Raman	PCA
	Almond oil (n = 11)	CAO1	spectroscopy	PLS-DA
		CAO11		
	Walnut oil (n = 5)	CWO1		
Lab-produced cold-pressed oils	Black cumin oil (n = 12)	CPBCO1	GC	ANOVA
		CPBCO12	Raman	PCA
	Almond oil (n = 12)	CPAO1	spectroscopy	PLS-DA
		CPAO12		
	Walnut oil (n = 9)	CPWO1		
Adulterated cold-pressed oils	Black cumin oil	CPBCO25%	Raman	PCA
	adulteration with %25 sunflower oil	SO	spectroscopy	PLS-DA
	with %50 sunflower oil	CPBCO25%		
	with %25 corn oil	CO		
	with %50 corn oil	CPBCO50%		
	Almond oil	CO		
	adulteration with %25 sunflower oil	CPAO25%		
	with %50 sunflower oil	SO		
	with %25 corn oil	CPAO50%		
	with %50 corn oil	SO		
	Walnut oil	CPAO50%		
	adulteration with %25 sunflower oil	CO		
	with %50 sunflower oil	CPWO25%		
	with %25 corn oil	SO		
	with %50 corn oil	CPWO50%		
		SO		
		CPWO25%		
		CO		
		CPWO50%		
		CO		

2.3. Instruments and data collection

2.3.1. Raman spectroscopy measurements

Raman spectra of the oil samples were obtained using a DeltaNu Examiner Raman Microscopy system (DeltaNu Inc., Laramie, WY, USA) with a 785 nm laser source and a cooled charge-coupled device (CCD at 0 °C) detector. Measurements were performed at room temperature. The oil sample (200 µL) was placed into a sealed glass vial and put in the Raman spectroscopy system. The obtained spectra were in the range of 200–2000 cm⁻¹ at a resolution of 2 cm⁻¹. Constant experiment parameters were set as follows: integration time of 15 s and 100 mW laser power. All measurements were carried out in triplicate for each sample.

2.3.2. GC-FID analysis

The fatty acid profiles of the oil samples were determined as fatty acid methyl esters (FAMES) by using GC-FID. The AOCS method was used for the preparation of the FAMES (AOCS, 1997). The analysis of the oil samples was performed using a Trace GC (ThermoScientific, Thermo Fisher Scientific, Inc., Waltham, MA, USA) equipped with a flame ionization detector. A capillary column (TR-WaxMS, Thermo Fisher Scientific, Inc., Waltham, MA, USA) with a 60 m × 0.25 mm internal diameter and 0.25 µm film thickness was used for the separation. The injection volume was 1 µL. The samples were injected in the split mode with a split ratio of 1:20. The temperature of the GC oven was programmed to 90 °C for 1 min then to increase by 6 °C/min to a final temperature of 230 °C, at which point the samples were held for 9 min. The injector and detector temperatures were 240 and 260 °C, respectively. The flow rate of helium carrier gas was 2 mL/min. FAMES were identified by comparison of retention times with authentic standards (Supelco 37 comp. FAME mix). The obtained results were expressed as a

percentage (% wt/wt) of all detected fatty acids by using the percentage of chromatographic peak area. The analysis was performed in duplicate for each sample.

2.4. Data processing

Stand-alone Chemometrics Software (Version Solo, 6.5 for Windows 7, Eigenvector Research Inc., Wenatchee, WA, USA) was used for chemometric analyses of Raman spectroscopic data. For data analyses, PCA and PLS-DA were chosen as unsupervised and supervised techniques, respectively.

An explorative study of the collected data was performed using PCA. Spectral residuals (Q residual values) were determined with PCA for each sample at a 95% confidence level. The collected data was split into calibration and validation sets by employing the Kennard-Stone approach provided by the software (Hirri, Bassbasi, Platikanov, Tauler, & Oussama, 2016). The calibration set was built with 2/3 of the samples from each class. The remaining 1/3 of the samples were used as the validation set. All models were validated with the Venetian Blinds cross-validation procedure. Apart from these, external data sets were used to develop prediction (test) models.

PLS-DA models were constructed for two different purposes; which are i) determination of cold-pressed oils adulterated with corn and sunflower oils, and ii) discrimination of cold-pressed and commercial oil samples. For the latter, the data belong to commercial oil samples were loaded as the external validation data set. The number of latent variables (LV) to be used in PLS-DA models was selected according to the point where class error values were minimized for calibration and cross-validation models. The effects of different preprocessing methods (e.g. mean centering, SNV, smoothing, and derivative) on the performance of the developed models were estimated based on root mean squared error of calibration (RMSEC), cross-validation (RMSECV), and prediction (RMSEP) models. The most reliable parameter that shows the future performance of the developed calibration model is the RMSEP value which is obtained by testing the model with a new data set that the calibration model had never seen before. In the absence of an external data set, the RMSECV value should be evaluated. RMSECV value is obtained by repeatedly removing and re-adding each data from the current data set. When a sample is removed from the data set, a calibration model is created with the remaining samples, and the value of the extracted sample is estimated by this established calibration model. The RMSEC value shows the compatibility of the calibration model with the calibration data set. The least informative value among the three values is the RMSEC value which is defined as an optimistic prediction of model performance (Bouveresse, 2007).

Sensitivity and specificity terms are described as the ability of the PLS-DA model to predict true positive values as positive and true negative values as negative (Feldsine, Abeyta, & Andrews, 2002; Trullols, Ruisánchez, & Rius, 2004). In the evaluation of the results of the PLS-DA model, confusion matrix values consisting of true positive (TP), false positive (FP), true negative (TN), and false negative (FN) ratios should be taken into consideration. These ratios were determined as previously reported (Feldsine et al., 2002). Class error (CE), sensitivity (Sens), and specificity (Spec) values are the other indices giving hints about the performance of the developed models. Sensitivity is the model's ability to predict known positives as positive. Specificity, on the other hand, is the ability of the model to predict known negatives as negative (Trullols et al., 2004).

Sensitivity values for calibration (Cal-Sens), cross-validation (CV-Sens), and prediction models (Pred-Sens) and specificity values for calibration (Cal-Spec), cross-validation (CV-Spec), and prediction models (Pred-Spec) were reported. Class error values for calibration (Cal-CE), cross-validation (CV-CE), and prediction models (Pred-CE) were reported. All of these values were tabulated as performance parameters of the developed models. Confusion matrices and confusion tables were created. The results of PLS-DA models were reported with

the class prediction member, probability, and PLS-DA score plots.

GC results were subjected to analysis of variance (ANOVA) and Duncan multiple comparison by using the statistical software SPSS 18.0 for Windows. The data obtained from the study were given as mean $\pm$ standard error (SE), and the level of confidence was 95% for all statistical analyses.

3. Results and discussion

3.1. Raman spectra of the cold-pressed oil samples

Raman spectral fingerprints of lab-produced cold-pressed almond, black cumin, and walnut oil samples were obtained. Characteristic Raman bands with different intensities were observed for the produced oil samples, and the related results are given in Fig. 1 a. As one can see from this figure for all three oil samples, broad Raman bands with low intensities around 247, 373, and 403 cm^{-1} were obtained. These bands were attributed to the skeletal mode, CCC deformations of the aliphatic chain, and C=C-C out of plane deformations of the olefinic groups, respectively (Machado, De Carvalho, Otero, & Marques, 2012). The other characteristic bands were assigned as follows. The bands located around 602 and 726 cm^{-1} were attributed to out of plane deformations of olefinic CH and OH groups, 870 and 918 cm^{-1} to various symmetric

CCC stretches, 972 cm^{-1} to = C-H out of plane deformation, 1083 cm^{-1} to CCC stretching, 1265 cm^{-1} to the = C-H symmetric rock of an unconjugated cis double bond, 1304 cm^{-1} to CH_2 twisting, 1441 cm^{-1} to CH_2 scissoring, 1656 cm^{-1} to cis double bond C=C stretching, and 1751 cm^{-1} to C=O stretching of an ester (Afseth, Wold, & Segtnan, 2006; Sadeghi-Jorabchi, Wilson, Belton, Edwards-Webb, & Coxon, 1991; Sánchez-Alonso, Carmona, & Careche, 2012).

Fig. 1 b, c, and d show the comparison between the average Raman spectra of cold-pressed and commercial oil samples. Similar fingerprint spectra with similar band intensities were obtained for the cold-pressed and commercial samples of almond and black cumin oils. On the other hand, band intensities of cold-pressed walnut oil were relatively higher than that of commercial walnut oil. These results have revealed the need for advanced data analysis techniques to discriminate between lab-produced and commercial oil samples by using Raman spectroscopy.

In recent years, the interest in searching for the benefits of oils obtained through cold press extraction technique has increased considerably. Cold pressing of oil seeds results in high-quality oils containing a vast amount of essential phytonutrients (Cecchi et al., 2019; Faugno et al., 2019; Teh & Birch, 2013; Wongwaiwech, Weerawatanakorn, Tharatha, & Ho, 2019). However, this popularity has inevitably brought about the adulteration of these high-quality oils.

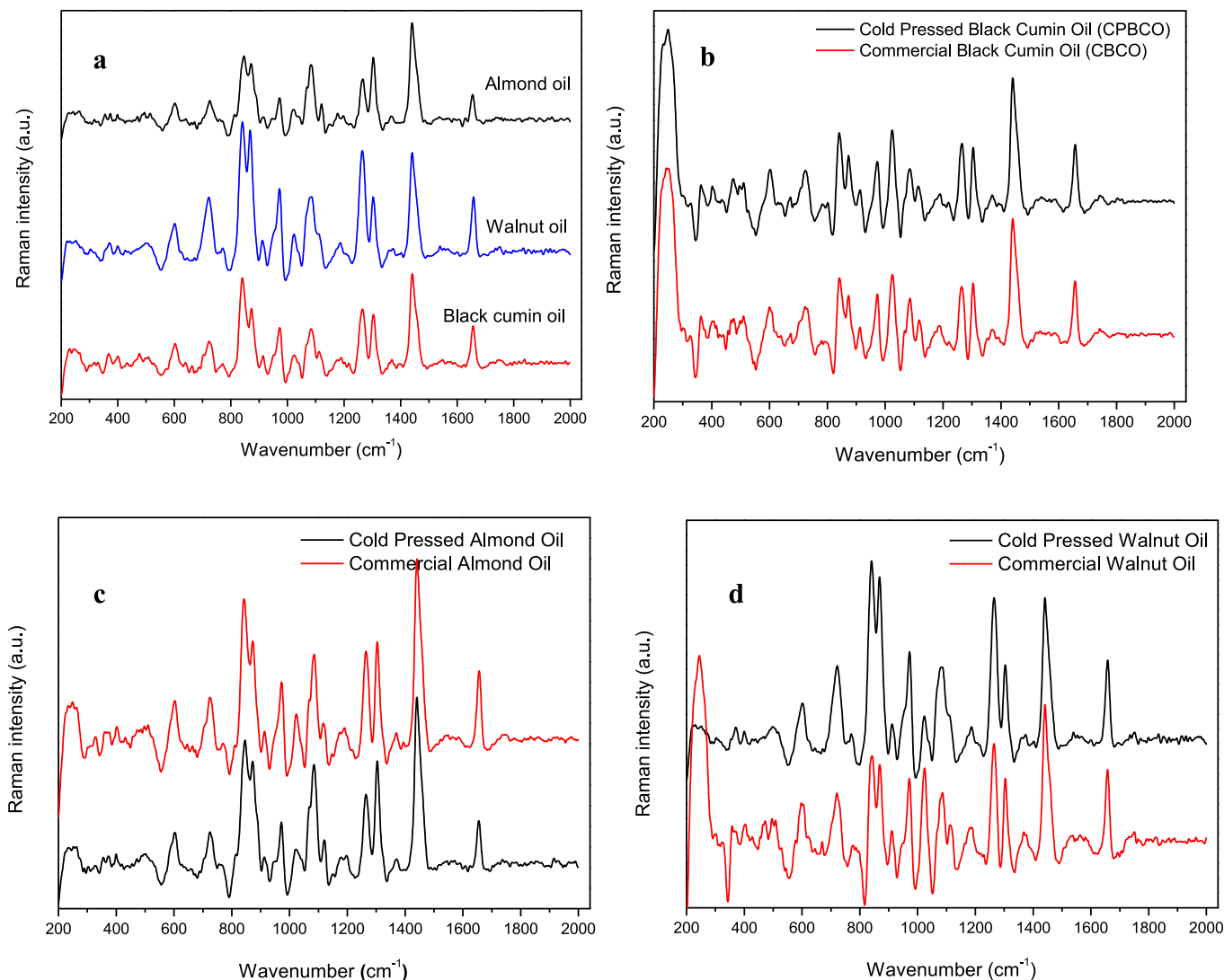


Fig. 1. Raman spectra of a) lab-produced cold-pressed oils and comparison between lab-produced and commercial cold-pressed oils b) BCO, c) AO, and d) WO.

3.2. GC analyses of the cold-pressed oil samples

Most of the physicochemical and nutritional properties of any type of oil are affected by the fatty acid profile. As expected, several factors including the variety of seed, regional variations, climatic conditions, harvesting, and storage time are responsible for the quality and composition of oil produced from agricultural sources (Ghazani,

García-Llatas, & Marangoni, 2014). Fatty acid profiles of almond, walnut, and black cumin oil samples, which were both produced in the laboratory and obtained from the market, are given in Table 2. According to the GC analysis of fatty acid methyl esters, palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 n-9), linoleic acid (C18:2 n-6), and γ -linolenic acid (C18:3 n-6) were found as primary fatty acids in almond, walnut, and black cumin oil samples, which together

Table 2

Fatty acid profiles of lab-produced and commercial cold pressed almond, walnut and black cumin oils.

Sample	C16:0 (Palmitic acid)	C18:0 (Stearic acid)	C18:1 n-9 (Oleic acid)	C18:2 n-6 (Linoleic acid)	C18:3 n-6 (γ -Linolenic acid)	C20:2 (Eicosadienoic acid)
CPAO1	6.6 $\pm$ 0.1 ^{a,b,c}	ND	71.1 $\pm$ 0.2 ^a	21.5 $\pm$ 0.0 ^a	ND	ND
CPAO2	6.4 $\pm$ 0.1 ^{c,d,e}	ND	69.8 $\pm$ 0.2 ^b	23.1 $\pm$ 0.1 ^k	ND	ND
CPAO3	6.5 $\pm$ 0.0 ^{b,c,d}	ND	69.8 $\pm$ 0.0 ^b	23.1 $\pm$ 0.1 ^k	ND	ND
CPAO4	6.8 $\pm$ 0.1 ^a	ND	63.0 $\pm$ 1.0 ^e	29.0 $\pm$ 0.0 ^f	ND	ND
CPAO5	6.6 $\pm$ 0.0 ^{a,b,c}	ND	70.2 $\pm$ 0.0 ^b	22.6 $\pm$ 0.2 ^l	ND	ND
CPAO6	6.5 $\pm$ 0.1 ^{b,c,d}	ND	69.9 $\pm$ 0.1 ^b	21.8 $\pm$ 0.1 ^{m,n}	ND	ND
CPAO7	6.4 $\pm$ 0.1 ^{c,d,e}	ND	70.0 $\pm$ 0.0 ^b	23.5 $\pm$ 0.1 ^k	ND	ND
CPAO8	6.7 $\pm$ 0.0 ^{a,b}	ND	71.1 $\pm$ 0.1 ^a	22.1 $\pm$ 0.1 ^m	ND	ND
CPAO9	6.5 $\pm$ 0.0 ^{b,c,d}	ND	68.9 $\pm$ 0.1 ^c	24.5 $\pm$ 0.1 ^j	ND	ND
CPAO10	6.5 $\pm$ 0.1 ^{b,c,d}	ND	65.5 $\pm$ 0.7 ^d	27.6 $\pm$ 0.4 ^h	ND	ND
CPAO11	6.6 $\pm$ 0.1 ^{a,b,c}	ND	65.0 $\pm$ 0.0 ^d	28.1 $\pm$ 0.1 ^g	ND	ND
CPAO12	6.6 $\pm$ 0.0 ^{a,b,c}	ND	68.3 $\pm$ 0.1 ^c	24.9 $\pm$ 0.1 ^l	ND	ND
CAO1	6.2 $\pm$ 0.0 ^{e,f}	2.1 $\pm$ 0.0 ^c	32.9 $\pm$ 0.0 ^h	52.7 $\pm$ 0.2 ^b	1.6 $\pm$ 0.0 ^b	ND
CAO2	6.2 $\pm$ 0.0 ^{e,f}	2.1 $\pm$ 0.0 ^c	33.7 $\pm$ 0.2 ^{g,h}	51.6 $\pm$ 0.2 ^d	1.8 $\pm$ 0.1 ^a	ND
CAO3	6.2 $\pm$ 0.1 ^{e,f}	2.1 $\pm$ 0.0 ^c	33.3 $\pm$ 0.1 ^h	52.2 $\pm$ 0.0 ^c	1.7 $\pm$ 0.0 ^{a,b}	ND
CAO4	6.2 $\pm$ 0.2 ^{e,f}	2.1 $\pm$ 0.1 ^c	33.5 $\pm$ 0.0 ^h	51.9 $\pm$ 0.0 ^{c,d}	1.7 $\pm$ 0.0 ^{a,b}	ND
CAO5	6.2 $\pm$ 0.0 ^{e,f}	2.1 $\pm$ 0.1 ^c	33.4 $\pm$ 0.0 ^h	52.0 $\pm$ 0.2 ^{c,d}	1.7 $\pm$ 0.0 ^{a,b}	ND
CAO6	6.2 $\pm$ 0.0 ^{e,f}	2.1 $\pm$ 0.0 ^c	33.4 $\pm$ 0.0 ^h	52.0 $\pm$ 0.1 ^{c,d}	1.7 $\pm$ 0.0 ^{a,b}	ND
CAO7	6.2 $\pm$ 0.0 ^{e,f}	2.1 $\pm$ 0.0 ^c	33.4 $\pm$ 0.0 ^h	52.0 $\pm$ 0.0 ^{c,d}	1.7 $\pm$ 0.0 ^{a,b}	ND
CAO8	6.2 $\pm$ 0.0 ^{e,f}	2.1 $\pm$ 0.1 ^c	33.4 $\pm$ 0.0 ^h	52.0 $\pm$ 0.0 ^{c,d}	1.7 $\pm$ 0.2 ^{a,b}	ND
CAO9	6.1 $\pm$ 0.1 ^f	3.8 $\pm$ 0.1 ^a	30.6 $\pm$ 0.0 ⁱ	56.8 $\pm$ 0.4 ^a	0.1 $\pm$ 0.0 ^{d,e}	ND
CAO10	5.8 $\pm$ 0.1 ^g	3.1 $\pm$ 0.0 ^b	39.1 $\pm$ 0.1 ^f	46.9 $\pm$ 0.1 ^e	1.1 $\pm$ 0.1 ^c	ND
CAO11	6.3 $\pm$ 0.0 ^{d,e,f}	3.2 $\pm$ 0.0 ^b	34.3 $\pm$ 0.2 ^g	52.7 $\pm$ 0.0 ^b	0.2 $\pm$ 0.0 ^d	ND
CPWO1	6.6 $\pm$ 0.0 ^{b,c}	2.4 $\pm$ 0.0 ^b	18.2 $\pm$ 0.0 ^d	60.0 $\pm$ 0.0 ^f	12.8 $\pm$ 0.1 ^{c,d}	ND
CPWO2	6.7 $\pm$ 0.2 ^b	2.5 $\pm$ 0.0 ^b	14.1 $\pm$ 0.0 ⁱ	64.3 $\pm$ 0.3 ^b	12.4 $\pm$ 0.0 ^{d,e}	ND
CPWO3	5.7 $\pm$ 0.0 ^e	2.6 $\pm$ 0.1 ^b	12.6 $\pm$ 0.2 ^j	64.7 $\pm$ 0.2 ^a	14.4 $\pm$ 0.0 ^a	ND
CPWO4	6.6 $\pm$ 0.1 ^{b,c}	2.4 $\pm$ 0.1 ^b	15.9 $\pm$ 0.4 ^{g,h}	62.4 $\pm$ 0.0 ^{c,d}	12.6 $\pm$ 0.0 ^{c,d}	ND
CPWO5	6.1 $\pm$ 0.0 ^d	2.5 $\pm$ 0.0 ^b	15.5 $\pm$ 0.0 ^h	62.3 $\pm$ 0.0 ^{c,d}	13.6 $\pm$ 0.4 ^b	ND
CPWO6	6.5 $\pm$ 0.0 ^{b,c}	2.4 $\pm$ 0.0 ^b	18.0 $\pm$ 0.0 ^{d,e}	61.2 $\pm$ 0.2 ^e	12.4 $\pm$ 0.4 ^{d,e}	ND
CPWO7	6.5 $\pm$ 0.1 ^{b,c}	2.4 $\pm$ 0.1 ^b	16.7 $\pm$ 0.1 ^f	62.3 $\pm$ 0.0 ^{c,d}	12.0 $\pm$ 0.1 ^{e,f}	ND
CPWO8	6.8 $\pm$ 0.1 ^b	2.4 $\pm$ 0.1 ^b	16.0 $\pm$ 0.0 ^g	62.1 $\pm$ 0.1 ^d	12.8 $\pm$ 0.0 ^{c,d}	ND
CPWO9	6.6 $\pm$ 0.0 ^{b,c}	2.5 $\pm$ 0.1 ^b	17.1 $\pm$ 0.1 ^f	61.0 $\pm$ 0.0 ^e	13.0 $\pm$ 0.0 ^c	ND
CWO1	10.0 $\pm$ 0.0 ^a	3.7 $\pm$ 0.2 ^a	31.2 $\pm$ 0.0 ^b	48.5 $\pm$ 0.0 ^h	6.6 $\pm$ 0.0 ^j	ND
CWO2	6.5 $\pm$ 0.1 ^{b,c}	2.5 $\pm$ 0.0 ^b	19.7 $\pm$ 0.2 ^c	59.7 $\pm$ 0.2 ^f	11.6 $\pm$ 0.2 ^f	ND
CWO3	6.0 $\pm$ 0.0 ^d	2.5 $\pm$ 0.0 ^b	32.2 $\pm$ 0.0 ^a	52.1 $\pm$ 0.1 ^g	7.2 $\pm$ 0.2 ⁱ	ND
CWO4	6.4 $\pm$ 0.0 ^c	2.5 $\pm$ 0.0 ^b	17.6 $\pm$ 0.2 ^e	62.7 $\pm$ 0.0 ^c	10.8 $\pm$ 0.0 ^g	ND
CWO5	6.4 $\pm$ 0.1 ^c	2.5 $\pm$ 0.0 ^b	17.6 $\pm$ 0.1 ^e	62.0 $\pm$ 0.0 ^d	9.1 $\pm$ 0.1 ^h	ND
CPBCO1	12.0 $\pm$ 0.0 ^{e,f}	ND	23.1 $\pm$ 0.0 ^{h,i,j}	58.9 $\pm$ 0.0 ^{a,b}	ND	2.5 $\pm$ 0.0 ^a
CPBCO2	12.8 $\pm$ 0.4 ^a	ND	22.4 $\pm$ 0.2 ^l	58.2 $\pm$ 0.4 ^c	ND	2.2 $\pm$ 0.0 ^b
CPBCO3	12.5 $\pm$ 0.1 ^{a,b,c}	ND	23.6 $\pm$ 0.1 ^{f,g}	58.9 $\pm$ 0.2 ^{a,b}	ND	2.3 $\pm$ 0.1 ^{a,b}
CPBCO4	12.7 $\pm$ 0.0 ^a	ND	25.3 $\pm$ 0.0 ^e	58.2 $\pm$ 0.0 ^c	ND	2.4 $\pm$ 0.0 ^{a,b}
CPBCO5	12.1 $\pm$ 0.1 ^{c,d,e,f}	ND	23.4 $\pm$ 0.0 ^{g,h}	56.9 $\pm$ 0.0 ^d	ND	2.4 $\pm$ 0.0 ^{a,b}
CPBCO6	12.1 $\pm$ 0.2 ^{d,e,f}	ND	23.3 $\pm$ 0.1 ^{g,h,i}	57.2 $\pm$ 0.1 ^d	ND	2.3 $\pm$ 0.0 ^{a,b}
CPBCO7	12.0 $\pm$ 0.0 ^{e,f}	ND	24.0 $\pm$ 0.0 ^f	57.0 $\pm$ 0.0 ^d	ND	2.4 $\pm$ 0.0 ^{a,b}
CPBCO8	12.3 $\pm$ 0.1 ^{b,c,d,e}	ND	22.9 $\pm$ 0.1 ^{i,j,k}	58.3 $\pm$ 0.1 ^{b,c}	ND	2.3 $\pm$ 0.0 ^{a,b}
CPBCO9	12.6 $\pm$ 0.1 ^{a,b}	ND	22.7 $\pm$ 0.1 ^{j,k,l}	59.0 $\pm$ 0.2 ^a	ND	2.3 $\pm$ 0.1 ^{a,b}
CPBCO10	12.0 $\pm$ 0.0 ^{e,f}	ND	23.1 $\pm$ 0.0 ^{h,i,j}	58.6 $\pm$ 0.1 ^{a,b,c}	ND	2.3 $\pm$ 0.1 ^{a,b}
CPBCO11	12.5 $\pm$ 0.1 ^{a,b,c,d}	ND	22.6 $\pm$ 0.5 ^{k,l}	58.3 $\pm$ 0.4 ^c	ND	2.4 $\pm$ 0.0 ^{a,b}
CPBCO12	12.0 $\pm$ 0.0 ^{e,f}	ND	22.4 $\pm$ 0.1 ^l	58.9 $\pm$ 0.2 ^{a,b}	ND	2.3 $\pm$ 0.0 ^{a,b}
CBCO1	12.2 $\pm$ 0.0 ^{b,c,d,e}	3.2 $\pm$ 0.0 ^{c,d}	23.9 $\pm$ 0.0 ^f	54.4 $\pm$ 0.2 ^g	0.4 $\pm$ 0.0	2.4 $\pm$ 0.0 ^{a,b}
CBCO2	7.5 $\pm$ 0.1 ^l	3.3 $\pm$ 0.2 ^{b,c}	30.2 $\pm$ 0.0 ^d	54.9 $\pm$ 0.1 ^g	0.8 $\pm$ 0.0	0.7 $\pm$ 0.0 ^{c,d}
CBCO3	12.1 $\pm$ 0.0 ^{c,d,e,f}	3.2 $\pm$ 0.1 ^{c,d}	23.1 $\pm$ 0.1 ^{h,i,j}	56.2 $\pm$ 0.0 ^e	0.2 $\pm$ 0.0	2.5 $\pm$ 0.2 ^a
CBCO4	7.9 $\pm$ 0.1 ^{g,h}	3.5 $\pm$ 0.0 ^a	30.2 $\pm$ 0.2 ^d	53.6 $\pm$ 0.4 ^h	0.7 $\pm$ 0.0	0.8 $\pm$ 0.1 ^c
CBCO5	11.7 $\pm$ 0.2 ^f	3.1 $\pm$ 0.0 ^d	23.7 $\pm$ 0.0 ^{f,g}	55.5 $\pm$ 0.2 ^f	0.8 $\pm$ 0.0	2.4 $\pm$ 0.1 ^{a,b}
CBCO6	5.8 $\pm$ 0.0 ^j	1.9 $\pm$ 0.1 ^f	54.2 $\pm$ 0.2 ^a	24.9 $\pm$ 0.0 ^k	5.8 $\pm$ 0.0	0.4 $\pm$ 0.0 ^e
CBCO7	7.6 $\pm$ 0.2 ^{h,i}	2.6 $\pm$ 0.1 ^e	46.1 $\pm$ 0.0 ^b	33.8 $\pm$ 0.2 ^j	3.7 $\pm$ 0.0	0.6 $\pm$ 0.0 ^d
CBCO8	8.2 $\pm$ 0.0 ^g	3.4 $\pm$ 0.0 ^{a,b}	33.1 $\pm$ 0.0 ^c	49.8 $\pm$ 0.0 ⁱ	1.1 $\pm$ 0.0	0.7 $\pm$ 0.0 ^{c,d}
CO	10.9 $\pm$ 0.0	2.0 $\pm$ 0.1	33.5 $\pm$ 0.4	52.2 $\pm$ 0.0	1.1 $\pm$ 0.0	ND
SO	6.0 $\pm$ 0.1	3.3 $\pm$ 0.0	32.4 $\pm$ 0.6	57.7 $\pm$ 0.3	ND	ND

CPAO: lab-produced almond oil, CAO: commercial almond oil, CPWO: lab-produced walnut oil, CWO: commercial walnut oil, CPBCO: lab-produced black cumin oil, CBCO: commercial black cumin oil, CO: corn oil, SO: sunflower oil.

ND: none detected.

All determinations were carried out in duplicate and mean value $\pm$ standard error (SE) were reported.

Statistical analysis were applied separately for oil samples namely almond, walnut and black cumin oil. Different letters in the column indicate significant differences ($p < 0.05$).

comprised approximately 95% of the total detected fatty acids. However, C18:0 and C18:3 n-6 were not detected in almond and black cumin oils produced in the laboratory. Additionally, black cumin oil samples varied from the others with their eicosadienoic acid (C20:2) content. C18:1 n-9 content of almond oil samples produced in the laboratory changed at a range of 63.0–71.1%, which is compatible with the findings of previous studies (Hernandez, 2015; Madawalaa, Kochharb, & Duttaa, 2012; Özcan et al., 2020). However, the C18:1 n-9 content of commercial almond oil samples was significantly lower than that of the ones produced in the laboratory ($P < 0.05$). This finding might be explained with the extraction method used for production, storage conditions of the final product in the market, or possible fraudulent applications in production.

The GC results obtained from walnut oil samples were compatible with the literature (Sielicka, Malecka, & Purlan, 2014; Viera-Alcaide et al., 2019). All samples were high in C18:2 n-6 and C18:1 n-9, and C18:3 n-6 followed this fatty acid. In addition to this, 2 out of 5 commercial walnut oil samples showed significantly different fatty acid composition with relatively high C18:1 n-9 content as 31.2% and 32.2%. This difference could be explained with possible malpractices in the production of cold-pressed oils.

Almost all black cumin oil samples (except two) were high in C18:2 n-6 as expected (Mahgoub, Ramadan, & El-Zahar, 2013; Ramadan et al., 2012). Additionally, black cumin seed oil differed from the other seed oils studied in this research with its C20:2 content. Remarkably, the samples produced in the laboratory lacked C18:3 n-6. Similarly, C18:0

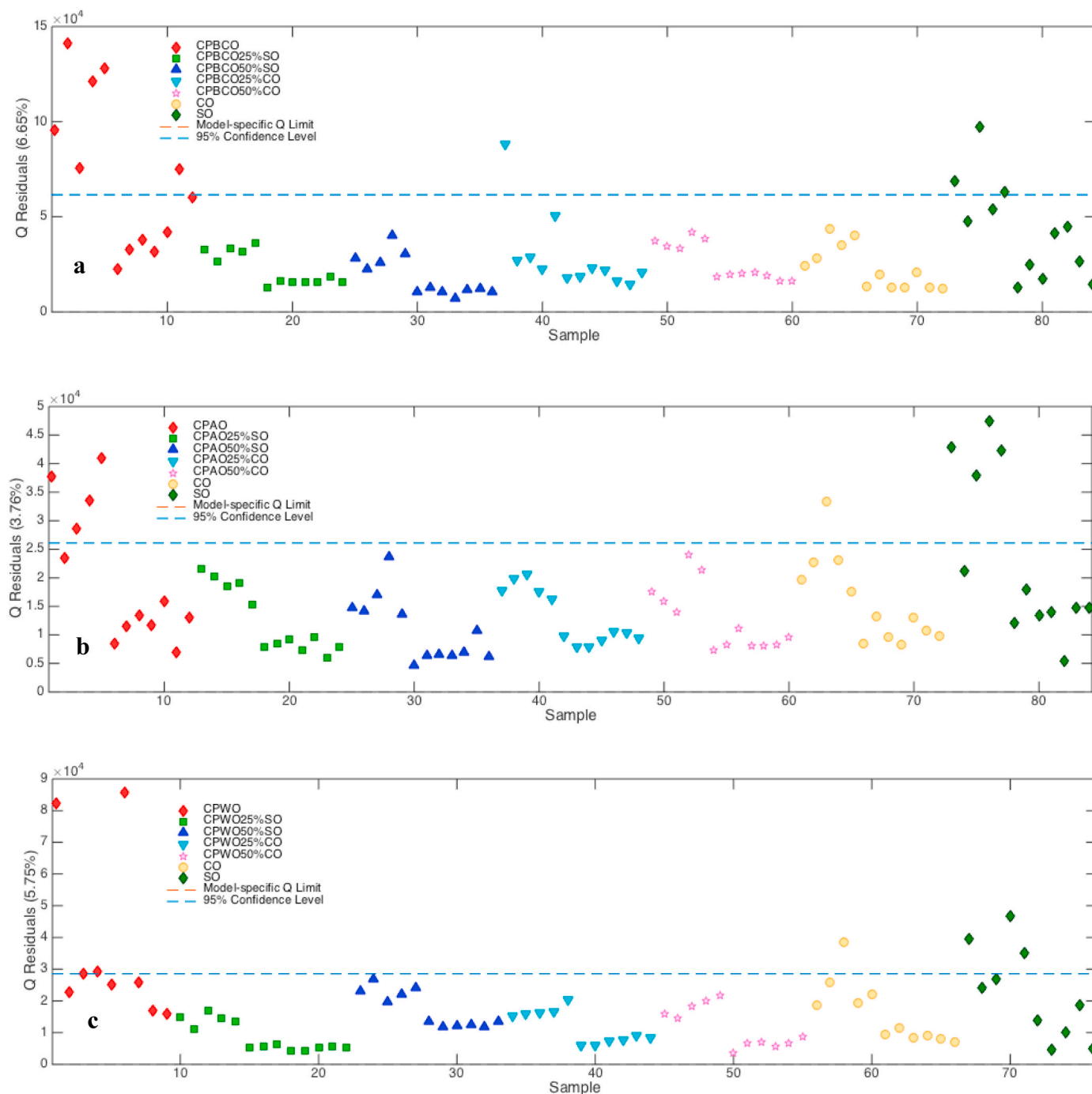


Fig. 2. Q Residuals for a-c) lab-produced and adulterated cold-pressed oil samples, d-f) commercial and lab-produced cold-pressed oil samples.

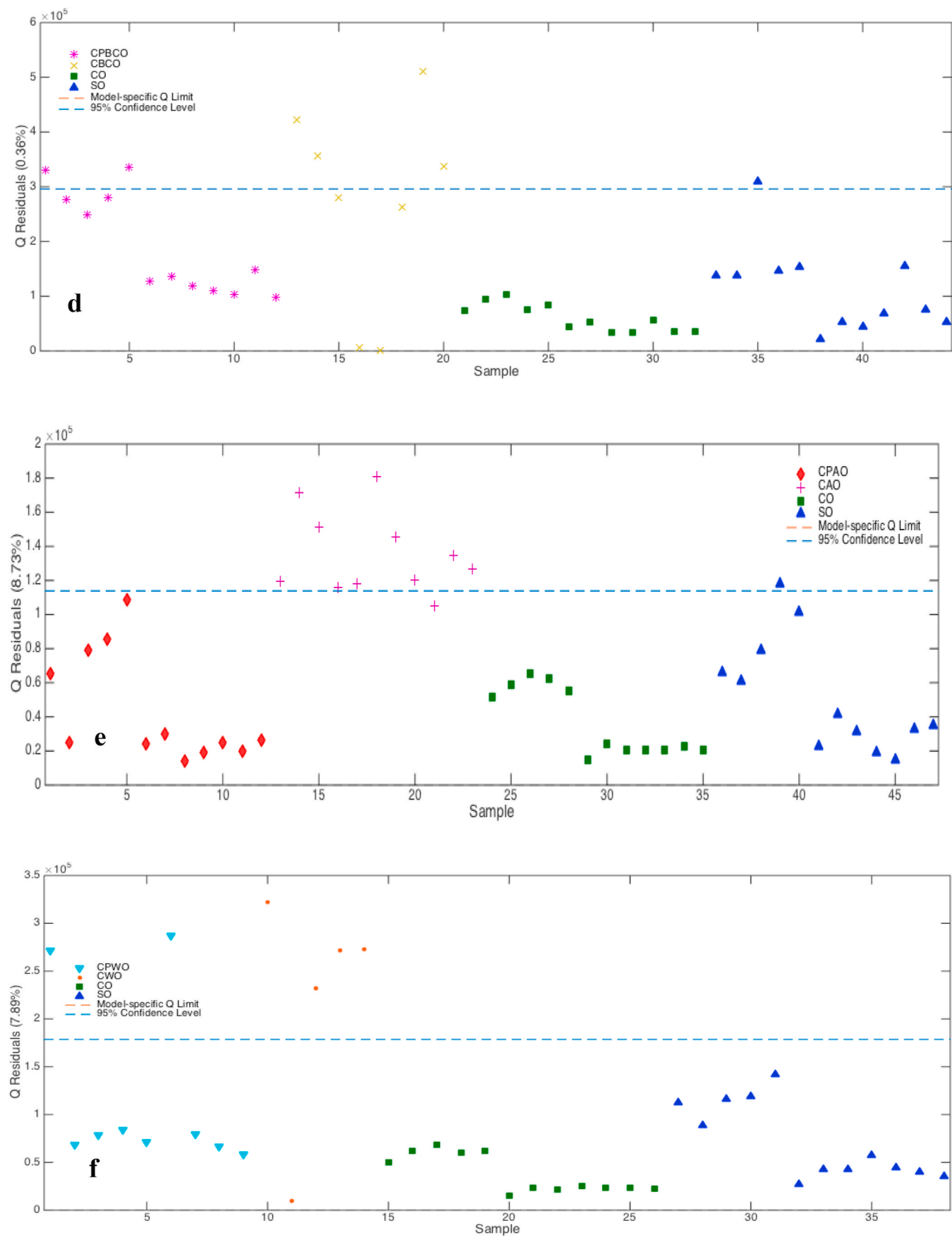


Fig. 2. (continued).

was not detected in the laboratory-produced samples. These findings were somewhat expected since production in the laboratory without applying any refining step may have caused impurities in the final product, and thus minor fatty acids could not be detected in GC analysis.

Overall, we speculated that the differences observed among the fatty acid compositions of oil samples produced in the laboratory, obtained commercially, or searched from the literature probably resulted from using different seed genotypes, applying different agricultural practices, using the oil extraction method, or any fraudulent activities performed in the commercial production.

3.3. Chemometric analyses of the cold-pressed oil samples

The use of chemometrics for reducing the dimensions of spectral data with minimum loss of information improves the capability of spectroscopic techniques in providing quantitative and qualitative information about the sample. Hence, principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were utilized in this study to analyze the data.

3.3.1. Classification of adulterated cold-pressed oil samples

Initially, the collected Raman spectroscopic data were analyzed using an unsupervised PCA model. Fig. 2a, b, and c show Q Residuals for pure and adulterated cold-pressed oil samples. First-order smoothing, the first derivative, and mean centering were used for all three PCA models. PCA model for CPBCO and its adulterated samples were built using five PCs, which explained 93.35% of the cumulative variance. A few samples belonging to CPBCO and SO classes presented comparatively larger spectral residuals. PCA models of CPAO and CPWO were built using five and six PCs, which explained 96.24% and 94.25% of the cumulative variance, respectively. The results obtained from these two models revealed that larger spectral residuals were observed for CPAO, CPWO, and SO samples.

PLS-DA models were constructed to discriminate adulterated samples from lab-produced cold-pressed oil samples. Details about the performance parameters of the PLS-DA models for CPBCO, CPAO, and CPWO adulterations are given in Table S1–S3.

PLS-DA model of CPBCO adulteration was constructed using six LVs and mean centering as the preprocessing. Cumulative variances of X and Y blocks were 97.31% and 79.79%. Hotelling T^2 and Q residuals values were found to be 97.31% and 2.69%. Sensitivity and specificity values

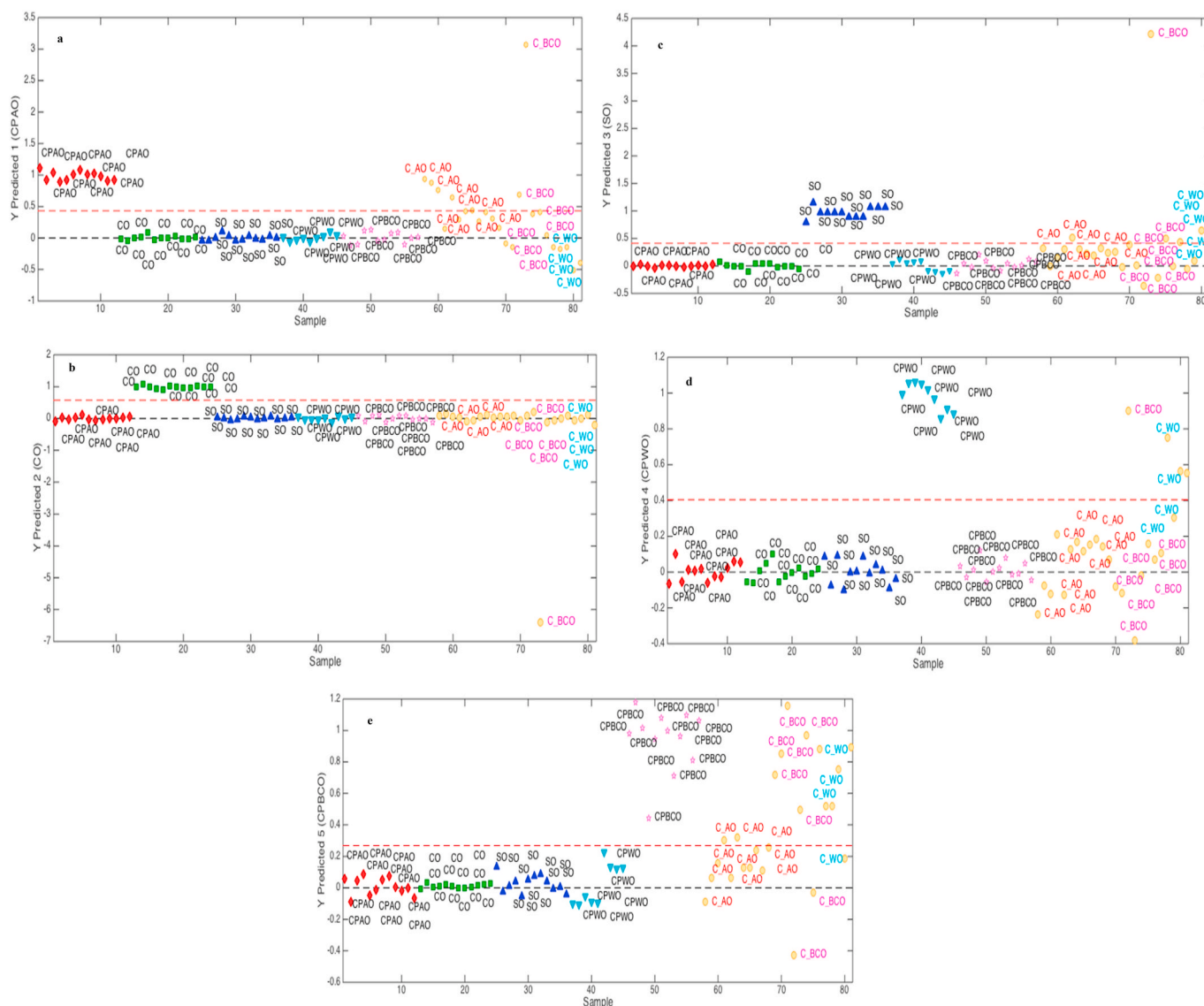


Fig. 3. PLS-DA score plots for discrimination of test samples (●) using calibration model consisting of a) Lab-produced cold-pressed almond oil; CPAO (◆) b) Corn oil; CO (■) c) Sunflower oil; SO (▲) d) Lab-produced cold-pressed walnut oil; WO (▼) and e) Lab-produced cold-pressed black cumin oil; CPBCO (*).

were 1.00 for all classes of the prediction model. RMSECV values were higher than RMSEP values, which were found to be at a range between 0.05 and 0.19. Class error values were zero for all classes of the prediction model. Score plots of PLS-DA prediction models are given in Fig. S1 a, b and c, where one can see that the Bayesian threshold values calculated by the software were presented with red horizontal dashed lines. The results obtained for CPBCO adulteration could be followed from Fig. 3a. Although large spectral residuals were observed for

CPBCO, all samples were predicted correctly by the model. The performance parameters, confusion matrix, and confusion table for this prediction model can be found in Table S1, S4 and Table S7.

For CPAO adulteration, eight LVs and mean centering were used. Cumulative variances were 98.23% and 93.28% for X and Y blocks. Hotelling T^2 and Q residuals values were 98.23% and 1.77%. As one can see from Table S2, sensitivity and specificity values were 1.00 for all classes. Larger spectral residuals for some CPAO and SO samples did not

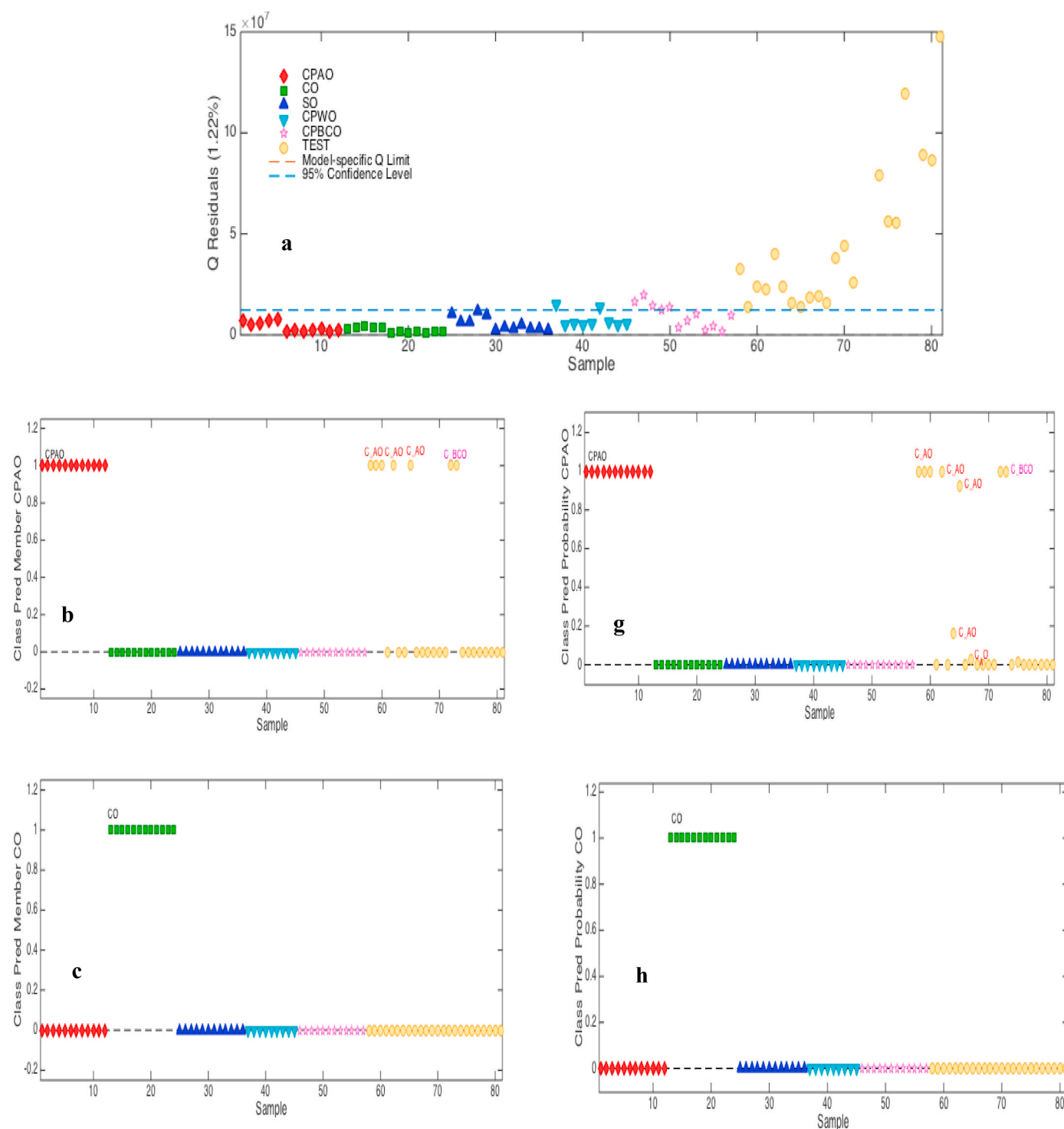


Fig. 4. Q Residuals (a), Class prediction member (b–f) and Probability plots (g–k) of PLS-DA model for discrimination of test samples (●) using calibration model constructed with lab-produced cold-pressed almond oil CPAO (◆); Corn oil; CO (■); Sunflower oil SO (▲); Lab-produced cold-pressed walnut oil WO (▼), and lab-produced cold-pressed black cumin oil CPBCO (*).

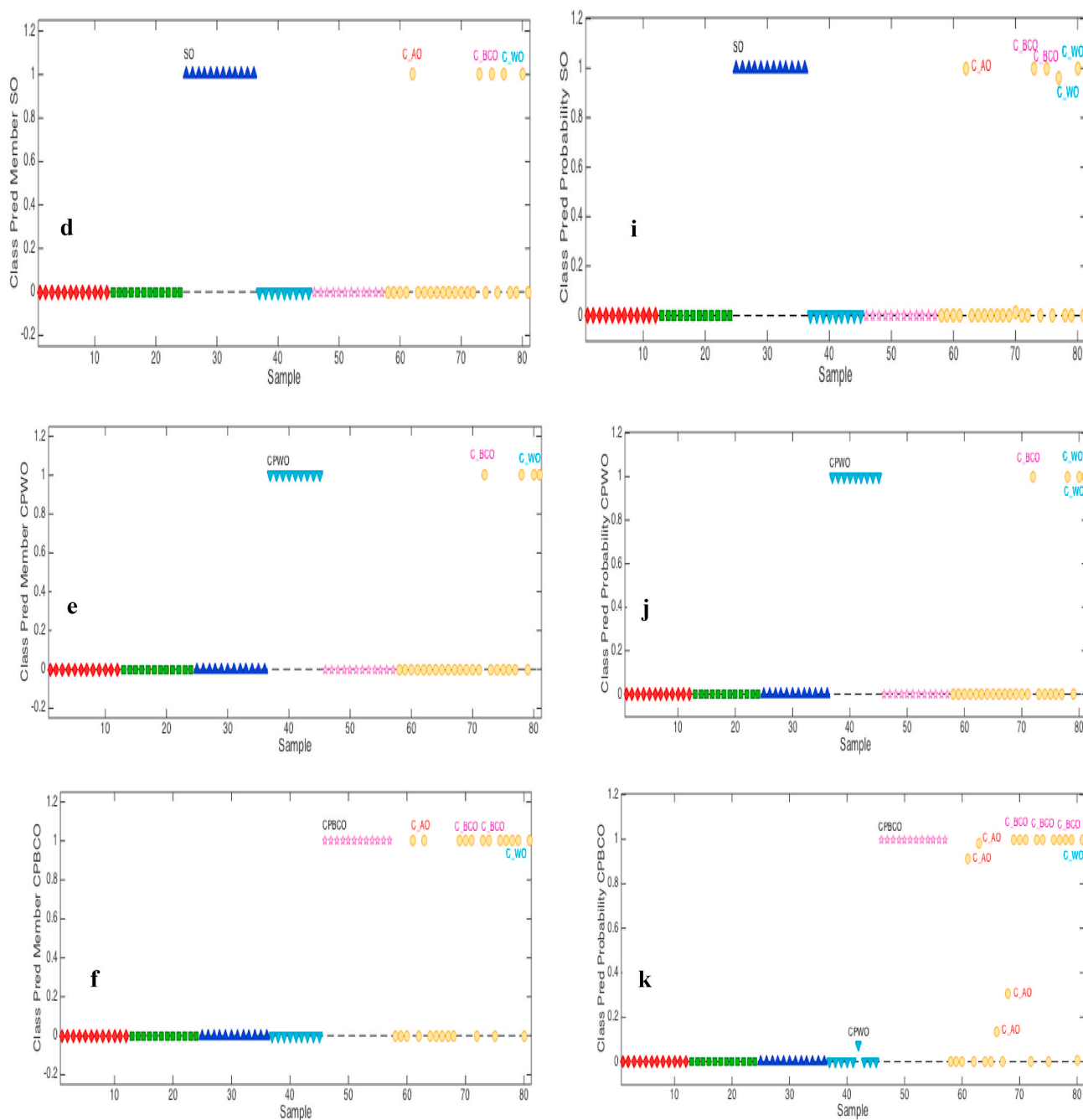


Fig. 4. (continued).

reduce the prediction ability of the model, and clear discrimination was observed, which can also be seen from Fig. 3b. The confusion matrix and confusion table for this prediction model can be found in Table S5 and Table S8. TP and TN values were 1.00 for all classes. Seven LVs and mean center were used to build the prediction model of CPWO adulteration. Cumulative variances were 97.41% and 87.67% for X and Y blocks. Hotelling T^2 and Q residuals values were 97.41% and 2.59%. Fig. 3c shows the PLS-DA score plots for this prediction model. From this figure, it can be seen that one CPWO sample was predicted as SO. This misclassification can be followed from both Pred-Spec and Pred-CE values of CPWO25%SO in Table S3. It also reduced the TP value of CPWO in the prediction model and increased the FP value of CPWO25% SO in the prediction model from Table S6. The confusion table in Table S9 also shows this misclassification. Loadings plots for PCA and PLS-DA adulteration models were given in Fig. S2.

3.3.2. Discrimination of cold-pressed and commercial oil samples

PCA model was developed to visualize cold-pressed and commercial oil samples. Fig. 2d, e, and f demonstrate Q Residuals for each sample. It can be seen in these figures that commercial samples presented more significant spectral residuals compared to the lab-produced cold-pressed oils. PCA models for discrimination between CPBCO, CPAO, and CPWO were built using eight, six, and five PCs, which explained 99.64%, 91.27%, and 92.11% of the cumulative variance, respectively.

PLS-DA model was constructed to discriminate commercial cold-pressed oils, which were loaded as an external validation set and titled as test samples. Seven LVs were used to build this model, which explained 98.78% and 96.51% of the cumulative variances corresponding to X and Y blocks, respectively. The mean centering was the only preprocessing used for this model. Fig. 4a shows that Q residuals for test samples were relatively higher than those for calibration samples.

Class prediction member plots are given in Fig. 4 b-f, and class prediction probability plots are presented in Fig. 4 g-k. PLS-DA score plots for the discrimination of test samples are given in Fig. 3. Table 3 summarizes the performance parameters of this PLS-DA model. As can be seen from the figures, test samples comprised eight CBCO, eleven CAO, and five CWO, while calibration samples were composed of twelve CPBCO, twelve CPAO, and nine CPWO. It makes the results more comprehensible when member plots and probability plots are evaluated simultaneously. Fig. 4b demonstrates that out of twenty-four test samples, five samples were assigned as CPAO, while there were a total of eleven CAO in the test data set. In Fig. 4g, the probability value of one more CAO sample was approximately 0.2 to be in the CPAO class while zero for the rest of the samples. It can be seen in Fig. 4c and h that none of the test samples were assigned to the CO class. On the other hand, in Fig. 4d and i, one CAO, two CBCO, and two CWO samples with probability values very close to 1.0 were assigned to the SO class. Fig. 4e and j show that out of five CWO, only three of them, along with one CBCO sample which has a very high probability value, were assigned to the CPWO class. In Fig. 4f and k, all CBCO samples were assigned to the CPBCO class with a 1.0 probability value. On the other hand, two CAO and 2 CWO samples were also assigned to the same class with relatively high probability values. From Table 3, the effects of these wrong assignments were monitored as decreases in specificity values and increases in class error values of prediction models for all classes.

In this study, the applicability of PLS-DA as one of the supervised chemometric methods has been investigated in terms of its ability to discriminate pure lab-produced oil samples from adulterated oil samples and to discriminate cold-pressed oil samples produced in laboratory conditions from commercial oil samples claimed to be produced using the cold-pressing technique. Discrimination of adulterated samples from pure cold-pressed oil samples was performed successfully with high sensitivity and specificity values of the prediction models.

The second aim of the study was to investigate the potential of Raman spectroscopy coupled with PLS-DA to discriminate three types of commercial oil samples by using one PLS-DA model. Although high sensitivity and specificity values were obtained for the prediction models, increasing the number of calibration samples may provide additional specificity, especially for CPBCO samples.

There is a limited number of studies reported on black cumin, almond, and walnut oils. Aroca-Santos et al. used visible spectroscopy with PLS and ANN to quantify binary and ternary mixtures containing extra virgin olive oil, soybean oil, and sweet almond oil (Aroca-Santos, Lastra-Mejías, Cancilla, & Torrecilla, 2019). Several researchers have assessed the suitability of different cultivars in terms of their potential as

edible oil resources (Rabadán, Pardo, Gómez, & Álvarez-Ortí, 2018; Sorkheh, Kiani, & Sofo, 2016; Wang et al., 2019) as well as different extraction techniques affecting the composition and functional properties of oils (Gao, Liu, Jin, & Wang, 2019a, 2019b; Gharibzadeh, Mousavi, Hamed, Rezaei, & Khodaiyan, 2013). There are studies employing vibrational spectroscopic techniques and chemometrics to analyze whole almonds (Cortés et al., 2018, 2019; Firmani, Bucci, Marini, & Biancolillo, 2019). Li et al. employed FTIR and fluorescence spectroscopy to determine adulteration of walnut oil by using walnut oils and potential adulterant oils, namely soybean, peanut, rapeseed, and sunflower oils were purchased from a local market (Li et al., 2015).

Susceptibility of cold-pressed oils to fraudulent practices has been emphasized in recent years. Several studies were conducted for determining the adulteration of cold-pressed oils. McDowell et al. employed low and high field ^1H NMR spectroscopy for the detection of commercial cold-pressed rapeseed oil adulteration with refined sunflower and rapeseed oils. High classification rates, 100% for single adulterant and 93% for multiple adulterants, were obtained using PLS-DA, SIMCA, and LDA (McDowell et al., 2018). Karuk Elmas et al. employed synchronous fluorescence spectroscopy (SFS) to detect commercial cold-pressed grape seed oil adulteration with refined soybean oil. SIMCA models provided a successful classification between commercial cold-pressed grape seed oils and other vegetable oils (Karuk Elmas et al., 2019). In another study by the same research group, a comparison was made between ATR-FTIR and SFS in terms of their ability to detect the adulteration of commercial cold-pressed black cumin seed oil. Based on the constructed SIMCA models, they emphasized the higher selectivity of SFS than ATR-FTIR to discriminate commercial cold-pressed black cumin seed oil from other vegetable oils (Arslan et al., 2019).

4. Conclusion

Although there are numerous studies on the detection of adulteration in edible oils in the current literature, there is a limited number of studies focusing particularly on cold-pressed oils. The interest in oils obtained by cold-pressing technique is rising rapidly in the world and the trend of healthy and conscious nutrition is gradually increasing. Within this context, it is of great importance to perform the analyses on cold pressed oil samples.

The present study highlighted the applicability of PLS-DA to discriminate lab-produced cold-pressed oil samples from adulterated oil samples and to discriminate cold-pressed oil samples produced in laboratory conditions from commercial oil samples claimed to be produced using the cold-pressing technique. Discrimination of adulterated samples from lab-produced cold-pressed oil samples was performed successfully, and high sensitivity and specificity values were achieved for the prediction models. Raman spectroscopy coupled with PLS-DA also accomplished to discriminate the three types of commercial oil samples. Although high sensitivity and specificity values were obtained for the prediction models, we presume that increasing the number of calibration samples may provide additional specificity, especially for CPBCO samples.

Finally, Raman spectroscopy coupled with chemometrics enabled discrimination of adulterated samples from pure cold-pressed oil samples. This study demonstrates the capability of Raman spectroscopy for rapid authentication of cold-pressed oils, hence we propose that it can be implemented for routine analysis.

CRedit authorship contribution statement

Havva Tumay Temiz: Investigation, Formal analysis, Data curation, Writing – original draft, Visualization. **Serap Durakli Velioglu:** Writing – review & editing, Data curation, Methodology. **Kadir Gurbuz Guner:** Resources, Visualization, Writing – original draft. **Hasan Murat Velioglu:** Conceptualization, Investigation, Project administration, Funding acquisition, Writing – review & editing.

Table 3

Performance parameters of PLS-DA models for discrimination of test samples.

	CPAO	CO	SO	CPWO	CPBCO
Cal-Sens	1.00	1.00	1.00	1.00	1.00
Cal-Spec	1.00	1.00	1.00	1.00	1.00
CV-Sens	1.00	1.00	1.00	1.00	0.92
CV-Spec	1.00	1.00	1.00	1.00	1.00
Pred-Sens	1.00	1.00	1.00	1.00	1.00
Pred-Spec	0.71	1.00	0.79	0.83	0.50
Cal-CE	0.00	0.00	0.00	0.00	0.00
CV-CE	0.00	0.00	0.00	0.00	0.04
Pred-CE	0.15	0.00	0.10	0.08	0.25
RMSEC	0.06	0.06	0.08	0.06	0.11
RMSECV	0.08	0.08	0.09	0.07	0.17
RMSEP	0.77	1.31	0.91	0.33	0.54

Abbreviations: CPBCO (lab-produced cold pressed black cumin oil), CPAO (lab-produced cold pressed almond oil), CPWO (lab-produced cold pressed walnut oil), CO (corn oil), SO (sunflower oil), Cal-Sens (sensitivity of calibration model), Cal-Spec (specificity of calibration model), Cal-CE (class error of calibration model), CV-CE (class error of cross validation model), RMSEC (root mean squared error of calibration model), RMSECV (root mean squared error of cross validation model), RMSEP (root mean squared error of prediction model).

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2021.111479>.

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References

- Afseth, N. K., Wold, J. P., & Segtnan, V. H. (2006). The potential of Raman spectroscopy for characterisation of the fatty acid unsaturation of salmon. *Analytica Chimica Acta*. <https://doi.org/10.1016/j.aca.2006.05.013>
- Aparicio, R., & Aparicio-Rufz, R. (2000). Authentication of vegetable oils by chromatographic techniques. *Journal of Chromatography A*. [https://doi.org/10.1016/S0021-9673\(00\)00355-1](https://doi.org/10.1016/S0021-9673(00)00355-1)
- Aroca-Santos, R., Lastra-Mejías, M., Cancilla, J. C., & Torrecilla, J. S. (2019). Linear and non-linear quantification of extra virgin olive oil, soybean oil, and sweet almond oil in blends to assess their commercial labels. *Journal of Food Composition and Analysis*. <https://doi.org/10.1016/j.jfca.2018.09.010>
- Arslan, F. N., Akin, G., Karuk Elmas, Ş. N., Yılmaz, I., Janssen, H. G., & Kenar, A. (2019). Rapid detection of authenticity and adulteration of cold pressed black cumin seed oil: A comparative study of ATR-FTIR spectroscopy and synchronous fluorescence with multivariate data analysis. *Food Control*. <https://doi.org/10.1016/j.foodcont.2018.11.055>
- Arslan, F. N., & Çağlar, F. (2019). Attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy combined with chemometrics for rapid determination of cold-pressed wheat germ oil adulteration. *Food Analytical Methods*. <https://doi.org/10.1007/s12161-018-1368-x>
- Aydeniz, B., Güneşer, O., & Yılmaz, E. (2014). Physico-chemical, sensory and aromatic properties of cold press produced safflower oil. *JAOCs. Journal of the American Oil Chemists' Society*. <https://doi.org/10.1007/s11746-013-2355-4>
- Beattie, J. R., Bell, S. E. J., & Moss, B. W. (2004). A critical evaluation of Raman spectroscopy for the analysis of lipids: Fatty acid methyl esters. *Lipids*. <https://doi.org/10.1007/s11745-004-1245-z>
- Bouveresse, C. B. (2007). Handbook of near-infrared analysis. In *Handbook of near-infrared analysis* (3rd ed.). CRC Press. <https://doi.org/10.1201/9781420007374>
- Cecchi, L., Innocenti, M., Urciuoli, S., Arlorio, M., Paoli, P., & Mulinacci, N. (2019). In depth study of phenolic profile and PTP-1B inhibitory power of cold-pressed grape seed oils of different varieties. *Food Chemistry*. <https://doi.org/10.1016/j.foodchem.2018.07.140>
- Čolić, S., Zec, G., Natić, M., & Fotirić-Aksić, M. (2019). Almond (*Prunus dulcis*) oil. In M. Ramadan (Ed.), *Fruit oils: Chemistry and functionality*. Cham: Springer.
- Cortés, V., Talens, P., Barat, J. M., & Lerma-García, M. J. (2018). Potential of NIR spectroscopy to predict amygdalin content established by HPLC in intact almonds and classification based on almond bitterness. *Food Control*. <https://doi.org/10.1016/j.foodcont.2018.03.040>
- Cortés, V., Talens, P., Barat, J. M., & Lerma-García, M. J. (2019). Discrimination of intact almonds according to their bitterness and prediction of amygdalin concentration by Fourier transform infrared spectroscopy. *Postharvest Biology and Technology*. <https://doi.org/10.1016/j.postharvbio.2018.05.006>
- Deelstra, H., Burns, D. T., & Walker, M. J. (2014). The adulteration of food, lessons from the past, with reference to butter, margarine and fraud. In *European Food Research and Technology*. <https://doi.org/10.1007/s00217-014-2274-0>
- Faugno, S., Sannino, M., Pacifico, S., Cervelli, E., Stagno, F., & Assirelli, A. (2019). New insights in hemp seed phenols and polyphenols through uhplc-esi-qtof-ms/ms analysis. *European Biomass Conference and Exhibition Proceedings*.
- Feldsine, P., Abeyta, C., & Andrews, W. H. (2002). AOAC International methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis. *Journal of AOAC International*. <https://doi.org/10.1093/jaoac/85.5.1187>
- Firmani, P., Bucci, R., Marini, F., & Biancolillo, A. (2019). Authentication of "Avola almonds" by near infrared (NIR) spectroscopy and chemometrics. *Journal of Food Composition and Analysis*. <https://doi.org/10.1016/j.jfca.2019.103235>
- Gao, P., Cao, Y., Liu, R., Jin, Q., & Wang, X. (2019). Phytochemical content, minor-constituent compositions, and antioxidant capacity of screw-pressed walnut oil obtained from roasted kernels. *European Journal of Lipid Science and Technology*. <https://doi.org/10.1002/ejlt.201800292>
- Gao, P., Liu, R., Jin, Q., & Wang, X. (2019a). Comparative study of chemical compositions and antioxidant capacities of oils obtained from two species of walnut: *Juglans regia* and *Juglans sigillata*. *Food Chemistry*. <https://doi.org/10.1016/j.foodchem.2018.12.016>
- Gao, P., Liu, R., Jin, Q., & Wang, X. (2019b). Comparison of solvents for extraction of walnut oils: Lipid yield, lipid compositions, minor-component content, and antioxidant capacity. *Lebensmittel-Wissenschaft & Technologie*. <https://doi.org/10.1016/j.lwt.2019.04.100>
- Ge, F., Chen, C., Liu, D., & Zhao, S. (2014). Rapid quantitative determination of walnut oil adulteration with sunflower oil using fluorescence spectroscopy. *Food Analytical Methods*. <https://doi.org/10.1007/s12161-013-9610-z>
- Gharibzadeh, S. M. T., Mousavi, S. M., Hamed, M., Rezaei, K., & Khodaiyan, F. (2013). Evaluation of physicochemical properties and antioxidant activities of Persian walnut oil obtained by several extraction methods. *Industrial Crops and Products*. <https://doi.org/10.1016/j.indcrop.2012.11.040>
- Ghazani, S. M., García-Llatas, G., & Marangoni, A. G. (2014). Micronutrient content of cold-pressed, hot-pressed, solvent extracted and RBD canola oil: Implications for nutrition and quality. *European Journal of Lipid Science and Technology*. <https://doi.org/10.1002/ejlt.201300288>
- Hernandez, E. M. (2015). Specialty oils: Functional and nutraceutical properties. Functional and nutraceutical properties. In *Functional dietary lipids: Food formulation, consumer issues and innovation for health*. <https://doi.org/10.1016/B978-1-78242-247-1.00004-1>
- Hirri, A., Bassabi, M., Platikanov, S., Tauler, R., & Oussama, A. (2016). FTIR spectroscopy and PLS-DA classification and prediction of four commercial grade virgin olive oils from Morocco. *Food Analytical Methods*. <https://doi.org/10.1007/s12161-015-0255-y>
- Jee, M. (2002). *Adulteration and authentication of oils and fats: An overview*. CRC Press. CRC Press.
- Karuk Elmas, Ş. N., Arslan, F. N., Akin, G., Kenar, A., Janssen, H. G., & Yılmaz, I. (2019). Synchronous fluorescence spectroscopy combined with chemometrics for rapid assessment of cold-pressed grape seed oil adulteration: Qualitative and quantitative study. <https://doi.org/10.1016/j.talanta.2018.12.026>
- Li, B., Wang, H., Zhao, Q., Ouyang, J., & Wu, Y. (2015). Rapid detection of authenticity and adulteration of walnut oil by FTIR and fluorescence spectroscopy: A comparative study. *Food Chemistry*. <https://doi.org/10.1016/j.foodchem.2015.02.079>
- Lutterodt, H., Luther, M., Slavin, M., Yin, J. J., Parry, J., Gao, J. M., et al. (2010). Fatty acid profile, thymoquinone content, oxidative stability, and antioxidant properties of cold-pressed black cumin seed oils. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology*. <https://doi.org/10.1016/j.lwt.2010.04.009>
- Machado, N. F. L., De Carvalho, L. A. E. B., Otero, J. C., & Marques, M. P. M. (2012). The autooxidation process in linoleic acid screened by Raman spectroscopy. *Journal of Raman Spectroscopy*. <https://doi.org/10.1002/jrs.4121>
- Madawalaa, S. R. P., Kochhar, S. P., & Dutta, P. C. (2012). Lipid components and oxidative status of selected specialty oils. *Grasas Y Aceites*. <https://doi.org/10.3989/gya.083811>
- Mahgoub, S. A., Ramadan, M. F., & El-Zahar, K. M. (2013). Cold pressed nigella sativa oil inhibits the growth of foodborne pathogens and improves the quality of domiati cheese. *Journal of Food Safety*. <https://doi.org/10.1111/jfs.12078>
- McDowell, D., Osorio, M. T., Elliott, C. T., & Koidis, A. (2018). Detection of refined sunflower and rapeseed oil addition in cold pressed rapeseed oil using mid infrared and Raman spectroscopy. *European Journal of Lipid Science and Technology*. <https://doi.org/10.1002/ejlt.201700472>
- Nurruhidayah, A. F., Che Man, Y. B., Al-Kahtani, H. A., & Rohman, A. (2011). Application of FTIR spectroscopy coupled with chemometrics for authentication of Nigella sativa seed oil. *Spectroscopy*. <https://doi.org/10.3233/SPE-2011-0509>
- Özcan, M. M., Al Juhaime, F., Ghafoor, K., Babiker, E. E., & Özcan, M. M. (2020). Characterization of physico-chemical and bioactive properties of oils of some important almond cultivars by cold press and soxhlet extraction. *Journal of Food Science & Technology*. <https://doi.org/10.1007/s13197-019-04128-3>
- Prescha, A., Grajzer, M., Dedyk, M., & Grajeta, H. (2014). The antioxidant activity and oxidative stability of cold-pressed oils. *JAOCs, Journal of the American Oil Chemists' Society*. <https://doi.org/10.1007/s11746-014-2479-1>
- Rabadán, A., Pardo, J. E., Gómez, R., & Álvarez-Ortí, M. (2018). Evaluation of physical parameters of walnut and walnut products obtained by cold pressing. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology*. <https://doi.org/10.1016/j.lwt.2018.01.061>
- Ramadan, M. F., Asker, M. M. S., & Tadros, M. (2012). Antiradical and antimicrobial properties of cold-pressed black cumin and cumin oils. *European Food Research and Technology*. <https://doi.org/10.1007/s00217-012-1696-9>
- Rodríguez, S. D., Gagneten, M., Farroni, A. E., Percibaldi, N. M., & Buera, M. P. (2019). FT-IR and untargeted chemometric analysis for adulterant detection in chia and sesame oils. *Food Control*. <https://doi.org/10.1016/j.foodcont.2019.05.025>
- Rohman, A., & Ariani, R. (2013). Authentication of nigella sativa seed oil in binary and ternary mixtures with corn oil and soybean oil using FTIR spectroscopy coupled with partial least square. *Science World Journal*. <https://doi.org/10.1155/2013/740142>
- Sadeghi-Jorabchi, H., Wilson, R. H., Belton, P. S., Edwards-Webb, J. D., & Coxon, D. T. (1991). Quantitative analysis of oils and fats by Fourier transform Raman spectroscopy. *Spectrochimica Acta Part A: Molecular Spectroscopy*. [https://doi.org/10.1016/0584-8539\(91\)80236-C](https://doi.org/10.1016/0584-8539(91)80236-C)
- Sánchez-Alonso, I., Carmona, P., & Careche, M. (2012). Vibrational spectroscopic analysis of hake (*Merluccius merluccius* L.) lipids during frozen storage. *Food Chemistry*. <https://doi.org/10.1016/j.foodchem.2011.10.047>

- Sielicka, M., Malecka, M., & Purlan, M. (2014). Comparison of the antioxidant capacity of lipid-soluble compounds in selected cold-pressed oils using photochemiluminescence assay (PCL) and DPPH method. *European Journal of Lipid Science and Technology*. <https://doi.org/10.1002/ejlt.201300356>
- Sorkheh, K., Kiani, S., & Sofo, A. (2016). Wild almond (*Prunus scoparia* L.) as potential oilseed resource for the future: Studies on the variability of its oil content and composition. *Food Chemistry*. <https://doi.org/10.1016/j.foodchem.2016.05.160>
- Sun, X., Zhang, L., Li, P., Xu, B., Ma, F., Zhang, Q., et al. (2015). Fatty acid profiles based adulteration detection for flaxseed oil by gas chromatography mass spectrometry. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology*. <https://doi.org/10.1016/j.lwt.2015.02.023>
- Teh, S. S., & Birch, J. (2013). Physicochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils. *Journal of Food Composition and Analysis*. <https://doi.org/10.1016/j.jfca.2013.01.004>
- Temiz, H. T., Tamer, U., Berkkan, A., & Boyaci, I. H. (2017). Synchronous fluorescence spectroscopy for determination of tahini adulteration. *Talanta*. <https://doi.org/10.1016/j.talanta.2017.02.044>
- Trullols, E., Ruisánchez, I., & Rius, F. X. (2004). Validation of qualitative analytical methods. *TRAC Trends in Analytical Chemistry*. [https://doi.org/10.1016/S0165-9936\(04\)00201-8](https://doi.org/10.1016/S0165-9936(04)00201-8)
- Uncu, O., Napiórkowska, A., Szajna, T. K., & Ozen, B. (2020). Evaluation of three spectroscopic techniques in determination of adulteration of cold pressed pomegranate seed oils. *Microchemical Journal*. <https://doi.org/10.1016/j.microc.2020.105128>
- Velioglu, S. D., Ercioglu, E., Temiz, H. T., Velioglu, H. M., Topcu, A., & Boyaci, I. H. (2016). Raman spectroscopic barcode use for differentiation of vegetable oils and determination of their major fatty acid composition. *JAOCs, Journal of the American Oil Chemists' Society*. <https://doi.org/10.1007/s11746-016-2808-7>
- Viera-Alcaide, I., Hamdi, A., Jiménez-Araujo, A., Rodríguez-Arcos, R., Espejo-Calvo, J. A., & Guillén-Bejarano, R. (2019). Nutritional composition and antioxidant activity of different walnut varieties (*Juglans regia* L.) from Nerpio (Spain) in comparison to commercial varieties. *Grasas Y Aceites*. <https://doi.org/10.3989/gya.0932182>
- Wang, W., Wang, H. L., Xiao, X. Z., & Xu, X. Q. (2019). Chemical composition analysis of seed oil from five wild almond species in China as potential edible oil resource for the future. *South African Journal of Botany*. <https://doi.org/10.1016/j.sajb.2018.11.009>
- Wongwaiwech, D., Weerawatanakorn, M., Tharatha, S., & Ho, C. T. (2019). Comparative study on amount of nutraceuticals in by-products from solvent and cold pressing methods of rice bran oil processing. *Journal of Food and Drug Analysis*. <https://doi.org/10.1016/j.jfda.2018.06.006>
- Wu, Z., Li, H., & Tu, D. (2015). Application of fourier transform infrared (FT-IR) spectroscopy combined with chemometrics for analysis of rapeseed oil adulterated with refining and purifying waste cooking oil. *Food Analytical Methods*. <https://doi.org/10.1007/s12161-015-0149-z>
- Xing, C., Yuan, X., Wu, X., Shao, X., Yuan, J., & Yan, W. (2019). Chemometric classification and quantification of sesame oil adulterated with other vegetable oils based on fatty acids composition by gas chromatography. *Lebensmittel-Wissenschaft & Technologie*. <https://doi.org/10.1016/j.lwt.2019.03.085>
- Yazgan Karacaglar, N. N., Bulat, T., Boyaci, I. H., & Topcu, A. (2019). Raman spectroscopy coupled with chemometric methods for the discrimination of foreign fats and oils in cream and yogurt. *Journal of Food and Drug Analysis*. <https://doi.org/10.1016/j.jfda.2018.06.008>
- Yildiz Tiryaki, G., & Ayvaz, H. (2017). Quantification of soybean oil adulteration in extra virgin olive oil using portable Raman spectroscopy. *Journal of Food Measurement and Characterization*. <https://doi.org/10.1007/s11694-016-9419-8>
- Zhao, X., Dong, D., Zheng, W., Jiao, L., & Lang, Y. (2015). Discrimination of adulterated sesame oil using mid-infrared spectroscopy and chemometrics. *Food Analytical Methods*. <https://doi.org/10.1007/s12161-015-0125-7>