



Cryogen free 60 MHz ^1H -NMR spectroscopy-based fingerprinting and chemometrics for the assessment of cold pressed black Cumin (*Nigella sativa* L.) seed oil adulteration

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Abstract

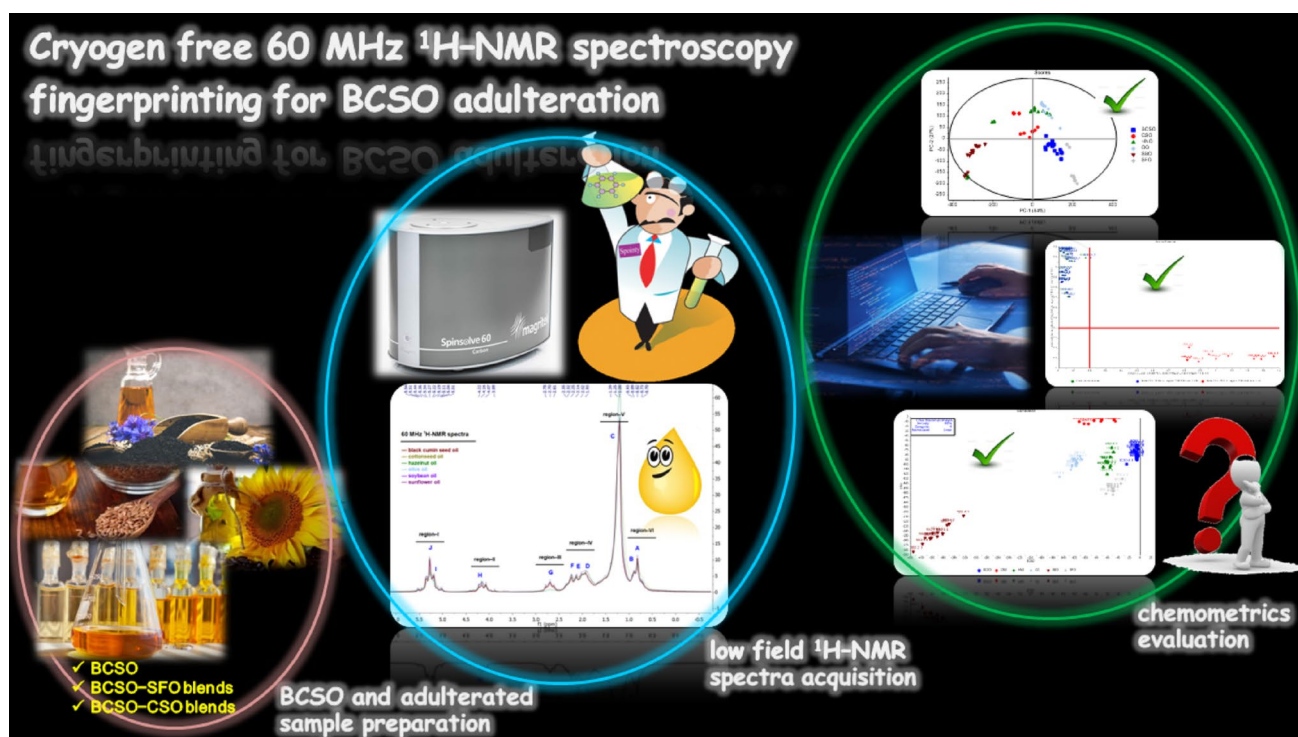
With current advances in the nuclear magnetic resonance (NMR) instrumentation, miniaturized cryogen-free low field spectrometers denote a technological breakup. This analytical strategy is utilized in wide range of scientific areas and provide an excellent approach to obtain data related for the structural characterization and fraud implementations in valuable edible oils. Herein, we aimed to study the feasibility and efficiency of cryogen-free benchtop NMR spectroscopy (60 MHz) with chemometrics for the detection of cold pressed black cumin (*Nigella sativa* L.) seed oil (BCSO) adulteration. This very simple and effortless procedure based on the glycerol backbone signals of triglycerides was highly adequate to detect adulteration qualitatively and quantitatively. The most popular chemometrics models of principle component analysis (PCA), hierarchical cluster analysis (HCA), soft independent modeling of class analogy analysis (SIMCA) and linear discriminant analysis (LDA) were built over the integrated data of NMR spectroscopy recorded from different oil samples. The partial least squares-regression (PLS-R) analysis models were also constructed to detect quantitative detection limits for the cheap refined cottonseed oils (CSOs) and sunflower oils (SFOs) in adulterated mixture sets ($n=144$). The samples were acceptably classified in their own types with an accuracy of 100% by the SIMCA and LDA model. The PLS-R results revealed that the detection limits of adulterant were 0.03% for BCSO-CSO ($R^2=0.9999\%$) and 0.13% for BCSO-SFO binary mixtures ($R^2=0.9999\%$), respectively. Consequently, the low-field ^1H -NMR spectroscopy allied with multivariate data analyses is expected, in the coming years, to become even more powerful analytical application for the detection of food frauds.

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Graphical abstract



Keywords Low-field NMR · Adulteration · Black Cumin seed oil · Chemometrics

Introduction

The black cumin (*Nigella sativa* L.) seed oil (BCSO) is a rich source of bioactive constituents and has many medicinal benefits. The BCSOs are commonly used as therapeutic form and well-known for their valuable medicinal effects including anti-oxidant, anti-tumor, anti-bacterial and anti-inflammatory effects [1–7]. Nowadays, the BCSOs are industrially processed by the screw pressing method known as “cold pressing extraction”, and they are used as a medicinal oil. The yield of oil from the cold pressing extraction process are rather low, and this cause quite high production costs and market prices. Hence, the increase in source and market demand of the cold pressed BCSOs have resulted in a gain of fraud implementations of them with inexpensive and valueless refined edible oils [7–9]. These illegal fraud practices might be potentially hazardous to the health of consumers like unwanted toxic effects or allergic reactions [3, 4, 10–13]. For this reason, the authentication and quality control strategies for the cold pressed BCSOs are of late extremely important topics in edible oil industry.

Nowadays, the adulteration of cold pressed BCSO is generally performed by the addition of low-cost and widely produced refined oils like soybean oil (SBO), cotton seed oil

(CSO) corn oil (CO) or sunflower oil (SFO) as an adulterant [4, 11, 13]. Their easy to find in markets, low price and difficulty in determining their presence due to their similar physicochemical and fatty acid (FA) composition characteristics to BCSO (mainly linoleic acid $\omega-6$, $C18:2$), making these oils attractive for the adulteration practices. To identify the presence of adulterants in the BCSO, the advance analytical methods like gas chromatography (GC) and high performance liquid chromatography (HPLC) are frequently applied; however, even they could be lack for the recognition of quality control and adulteration [1–3, 7]. Consequently, the development of new analytic methodologies is a challenging topic of search in literature.

Within this context, the conventional spectroscopy methods such as Fourier transform infrared spectroscopy (FTIR), fluorescence (FL) spectroscopy, Raman spectroscopy and mass spectroscopy (MS) allied with multivariate data analyses have been mostly developed [1–3, 7, 14, 15]. However, the accurate detection of adulterants could be difficult with the application of these methods, owing to the peak overlapping of compounds in similar structures. As promising and effective alternative to these methods, the NMR spectroscopy has recently been take attention for the adulteration searches in edible oil industry [16–19]. The high-field NMR method is revealed to be highly effective for the recognition

of routine quality control analyses and the adulteration of edible oils composed of several FAs within different ratios in triglycerides that prove their types [20]. However, the applications of high-resolution NMR spectroscopy are highly limited with the expensive technical and operation costs due to a superconducting magnet and nonstop cryogen consumption, as well as they require skilled analysts to use the instrument. The current progress in cryogen free benchtop NMR spectroscopy instruments has made this strategy a cost-effective and much more attractive approach [21, 22]. Hence, this technique is demonstrated through the routine fraud applications by combining of the chemometrics and the overlapping of target signals in spectral data could be effortlessly avoided [19, 23–33]. With regard to operation and rapid screening, benchtop low field NMR spectroscopy (60 MHz) has further in common with FTIR spectroscopy, than with high-field NMR approach [28, 30, 32, 34–39]. The ease of usage and the reasonable price of FTIR spectroscopy make it an attractive method particularly for the adulteration applications in edible oil sector [10, 36–39], and it provides a natural comparing with benchtop NMR spectroscopy. However, the chemical specificity detectable in low field NMR spectrum for any edible oil sample provides it a highly significant advantageous over the FTIR methods [32]. Another important point is that the information about fatty acids and their derivatives assigned by high-field NMR spectroscopy could be easily applied to similar low-field spectroscopy data/peaks [28, 30, 32, 34]. On the contrary, there is widespread overlap of the bands arising from the numerous vibrations of diverse chemical bonds in edible oil samples, making complete peak explanation hard even in the fingerprint region of FTIR spectroscopy [32]. Herein, the feasibility and efficiency of the cryogen free benchtop ^1H -NMR spectroscopy method for the authentication of cold pressed BCSOs was described. The qualitative chemometric analyses including SIMCA, HCA, LDA and PCA models, as well as the quantitative PLS-R analysis models were successfully applied over the integrated ^1H -NMR data. The qualification and quantification of the adulterants in adulterated cold pressed BCSO sets (*the mixtures of BCSO-CSO and BCSO-SFO*) was studied in detail.

Experimental section

General

The chemicals [*chloroform-d*, *KOH*, *methanol*, *hexane* and *fatty acid methyl esters mixture (C4–C24, FAME)*] were purchased from Sigma-Aldrich Inc. (St. Louis, Missouri, US) and VWR Chemicals BDH Inc. (West Chester, Pennsylvania, US). The mixture of FAMES as a standard reagent

was bought from the company of Supelco[®] Analytical Products (Merck KGaA, Darmstadt, Germany). The oil samples under study were supplied from the local stores (Karaman, Türkiye) with the highest sales possible and shelf authority, and their qualities were all certificated on their labels with their tolerable limits by the manufacturers. The cold pressed BCSOs (*BCSO-1 to BCSO-8*; $n = 8$), refined SBOs (*SBO-1 to SBO-5*; $n = 5$), refined hazelnut oil (HNO) (*HNO-1 to HNO-6*; $n = 6$), refined CSOs (*CSO-1 to CSO-5*; $n = 5$), refined SFOs (*SFO-1 to SFO-5*; $n = 5$) and virgin olive oil (VOO) (*VOO-1 to VOO-5*; $n = 5$) samples in different commercial brands were supplied. Afterwards, they were filtered by using 0.45 μm Nylon-membrane filter to get rid of possible contaminations.

The sample preparation procedure under study is presented in Supplementary Table 1. Four different brands of cold pressed BCSO samples (*BCSO-1, BCSO-2, BCSO-3 and BCSO-4*) were individually adulterated by blending with different brands of the refined SFO samples (*SFO-1 and SFO-2*; $n = 72$; 0–50%) and the refined CSO samples (*CSO-1 and CSO-2*; $n = 72$; 0–50%) (Supplementary Table 1). A total of 148 different samples of known origins and laboratory prepared binary mixtures were utilized to construct the chemometric models.

Acquisition of cryogen-free 60 mhz NMR spectra

A Spinsolve benchtop cryogen-free 60 MHz NMR spectroscopy instrument (Magritek GmbH, Aachen, Germany) was used to gather spectral data. The spectra were collected within the time of 10 min for each sample, and the scan number of 64 was applied for the measurements. 200 μL of oil sample was put into a standard NMR-tube (Aldrich ColorSpec, 5 mm diam. $\times$ 8 inc. size), and then 200 μL of chloroform-*d* [99.9%, 0.03% tetramethylsilane (TMS)] was added into the NMR tube [22, 30, 32], and then it was shaken by the vortex apparatus. The spectra were acquired at least in three replicates for the multivariate data analyses. The integration process of the NMR spectra was carried out by MestReNova v.12.0.0–20080 package (Mestrelab Res., Santiago de Compostela, Spain). The integrated ^1H -NMR data of oil samples were graphed by the OriginPro 9 (OriginLab, US) software.

Chemometrics

The raw ^1H -NMR data were exported in matrixes as Excel files (Office-365, Microsoft Corp., Redmond, WA, US), afterwards they were imported to the CAMO Unscrambler[®]X10.4 software package [40, 41]. Before the multivariate data analyses, the preprocessing applications were done over the raw spectral data (Supplementary Fig.

1b, c), and then the supervised (*LDA and SIMCA*), unsupervised (*HCA and PCA*) and regression (*PLS-R*) analyses were performed. The results were evaluated both over the spectral data of full and different chemical shift ranges (*NMR region-I to -VI*) symbolizing the compounds in tested samples (Table 1).

The PCA score plots were constructed by evaluating the spectra recorded in the spectral ranges of full region; 6.00–0.00 δ , region-I; 6.00–4.75 δ , region-II; 4.75–3.50 δ , region-III; 3.50–2.50 δ , region-IV; 2.50–1.75 δ , region-V; 1.75–1.00 δ and region-VI; 1.00–0.25 δ . The statistical performances of the developed PCA score plots were assessed based on important parameter of the eigen-values defining the total percentage of first two PCs. Similarly, LDA, SIMCA and HCA classification models were developed by evaluating the spectra obtained from the full and above-mentioned regions. The PLS-R plots were finally developed for the recognition of refined SFO or CSO adulterants in the cold pressed BCSO samples by studying the spectra obtained from the range of 6.00–0.00 δ . The procedure of “leave-one-out” based on cross-validation methodology [4,

42–44] was performed over the spectra. In the PLSR analysis, spectral data were divided into two groups as “calibration data set” (75% of samples) to create the model and “validation data set” (25% of samples) to test the validity of the model by the cross-validation method. To evaluate the performance of the developed calibration models and assess their diagnostic abilities; statistically significant parameters including limit of detection (LOD) in calibration sets namely root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV) and R-square values were calculated. The RMSECV was used as an internal indicator for the predictive ability of the models and small values of it are indicative of a superior prediction ability of the developed PLSR model. The value of RMSEC is also a statistical parameter how well the model predicts new samples and states the average error to be expected in future predictions when the PLSR model is applied to unknown samples. The R-square was utilized as an indicator of the models’ predictive ability [4, 42].

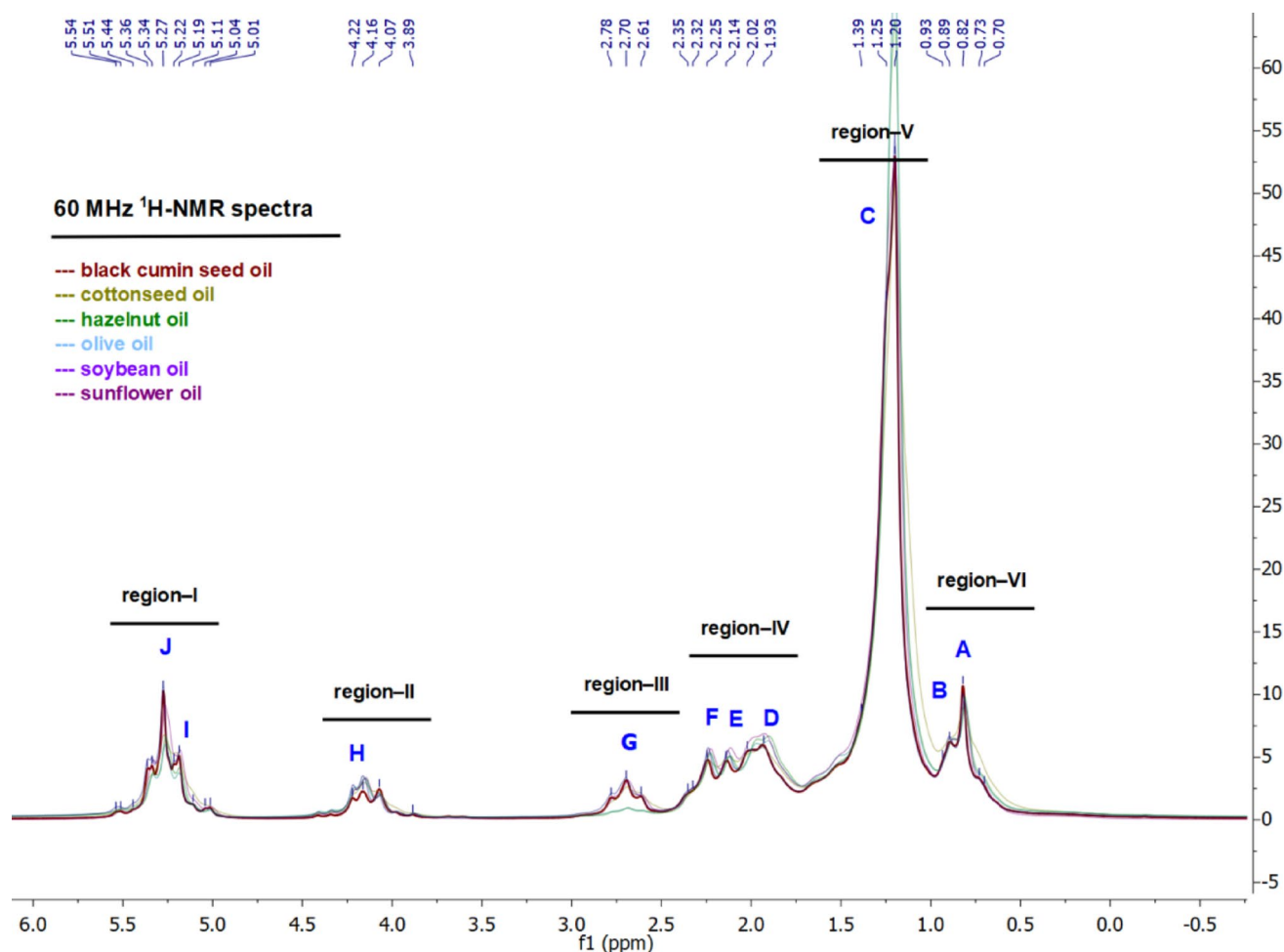


Fig. 1 Example of ^1H -NMR results gathered over the section of 6.00–0.00 δ for the BCSO and other edible oil samples

Table 1 The chemical shifts (δ) and functional groups in the spectral data of edible oils for the chemometric assessment

	δ range	Nominal δ	Compounds
Region-I	6.00–4.75 (<i>band-I</i>)	5.51	β -carotene
		5.27	$-\text{CH}=\text{CH}-$ (all unsaturated FAs) [†]
		5.22	$-\text{CH}-\text{OCO}-\text{R}$ (glycerol structure, triglycerides)
		5.19	$-\text{CH}-\text{OCO}-\text{R}$ (glycerol structure, triglycerides)
Region-II	4.75–3.50 (<i>band-II</i>)	5.06	$-\text{CH}-\text{OH}$ (1,3-diglycerides)
		4.47	$-\text{CH}_2-\text{OCO}-\text{R}$ sn-1,3 (1,3-diglycerides)
		4.39	$-\text{CH}_2-\text{OCO}-\text{R}$ (glycerol structure, triglycerides)
		4.25	$-\text{CH}_2-\text{OCO}-\text{R}$ (glycerol structure, triglycerides)
		4.16	$-\text{CH}_2-\text{OCO}-\text{R}$ (glycerol structure, triglycerides)
		4.07	$-\text{CH}_2-\text{OCO}-\text{R}$ sn-1,3 (1,3-diglycerides)
		3.89	$-\text{CH}_2-\text{OCO}-\text{R}$ sn-1,2 (1,2-diglycerides)
Region-III	3.50–2.50 (<i>band-III</i>)	2.79	$-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-$ (linoleic group)
		2.70	$-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-$ (linoleic group, UFAs)
Region-IV	2.50–1.75 (<i>band-IV</i>)	2.38	$-\text{CH}_2-\text{COO}-$ (all FA acyl chains)
		2.25	$-\text{CH}_2-\text{COO}-$ (all FA acyl chains)
		2.14	$-\text{CH}_2-\text{COO}-$ (all FA acyl chains)
		2.03	$-\text{CH}_2-\text{CH}=\text{CH}-$ (all UFAs, linoleic and linolenic groups)
		1.93	$-\text{CH}_2-\text{CH}=\text{CH}-$ (all UFAs, oleic groups)
Region-V	1.75–1.00 (<i>band-V</i>)	1.65	$-\text{CH}_2-\text{CH}_2-\text{COO}-$ (all FS acyl chains)
		1.55	$-\text{CH}_2-\text{CH}_2-\text{COO}-$ (all FA acyl chains)
		1.20	$-(\text{CH}_2)_n-$ (all FA acyl chains)
Region-VI	1.00–0.25 (<i>band-VI</i>)	0.89	$-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_3$ (all UFA acyl chains)
		0.82	$-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$ (all UFA, except the linoleic group)
		0.71	stigmastanol

FA fatty acid, SFA saturated fatty acid, UFA unsaturated fatty acid, PUFA polyunsaturated fatty acid, MUFA monounsaturated fatty acid

[†]Hydrogen bond indicated by the signal of $^1\text{H-NMR}$ spectra

Fatty acid composition analysis by GC-FID

The FA profiles of BCSOs and other studied oils were determined via a 7890 A model GC-FID system (Agilent Tech., Wilmington, DE, US). Before the analysis, the samples were converted to their FAMES according to base-catalyzed

esterification method [45, 46]. The analysis was performed on a HP88-cyanopropyl GC capillary column (0.2 $\mu\text{m} \times 0.25 \text{ mm i.d.} \times 100 \text{ m}$). The detector and injector compartments were 250 $^\circ\text{C}$, and the injection of 1 μL sample was injected to the system in split mode (100:1). The carrier gas was Helium at a flow rate of 1.0 mL min^{-1} . The oven temperature program was initially set at 45 $^\circ\text{C}$ (5 min), then it gradually raised to 175 $^\circ\text{C}$ at a rate of 13 $^\circ\text{C min}^{-1}$, kept it at 175 $^\circ\text{C}$ (10 min), and lastly it was ramped at a rate of 4 $^\circ\text{C min}^{-1}$ from 175 $^\circ\text{C}$ to 215 $^\circ\text{C}$, kept at 215 $^\circ\text{C}$ (35 min). The data were evaluated by using Chemstation B.03.02/2008 package. The amounts of FAs were identified by comparing the normalized peak areas to reference material (FAME mixture, Mix C4–C24), and all data were recorded in triplicate. The results for the FA contents were given as mean $\pm$ SD, and they were used as a quality control data of the studied oil samples.

Results and discussion

Low-field $^1\text{H-NMR}$ data interpretation

The low-field $^1\text{H-NMR}$ spectra of the BCSO and other oil samples are presented in Fig. 1. As seen in Fig. 1, owing to the decrease in field-strength of the instrument, the task of signals becomes more confused and the nuclei interaction (J coupling) of peaks couldn't clearly be visible in different edible oil samples. The low-field $^1\text{H-NMR}$ spectra of different edible oils are previously presented in the limited literature [23–28, 30–33], and the identification of peaks is possible as given in Table 1.

A typical low-field $^1\text{H-NMR}$ spectrum depicted the resonances related to both FAs and glycerol backbone in diverse spectral regions (*region-I* to *-VI*) (Fig. 1). The data could be evaluated into the 6 different spectral regions (*region-I*; 6.00–4.75 δ , *region-II*; 4.75–3.50 δ , *region-III*; 3.50–2.50 δ , *region-IV*; 2.50–1.75 δ , *region-V*; 1.75–1.00 δ and *region-VI*; 1.00–0.25 δ), which symbolize the identification of main peaks of compounds (Table 1). The $^1\text{H-NMR}$ spectra were collected by the signals of glycerol backbone in triglyceride molecules. Being that the triglyceride molecules of the oil samples under study are composed of different type of FAs such as linoleic (C18:2), oleic (C18:1), stearic (C18:0) and palmitic (C16:0) acids [23–28, 30–33].

As seen in Fig. 1, the peaks **J** and **I** at 5.27 ppm and 5.19 ppm (*region-I*) symbolize the olefinic and one methine protons of the $-\text{CH}=\text{CH}-$ (UFAs) and $-\text{CH}-\text{OCO}-\text{R}$ (glycerol structure, triglycerides) groups, respectively. In the *region-II*, the peak **H** at 4.16 ppm corresponds to the methylene protons of $-\text{CH}_2-\text{OCO}-\text{R}$ (glycerol structure, triglycerides) groups. The peak **G** at 2.70 ppm (*region-III*) symbolizes

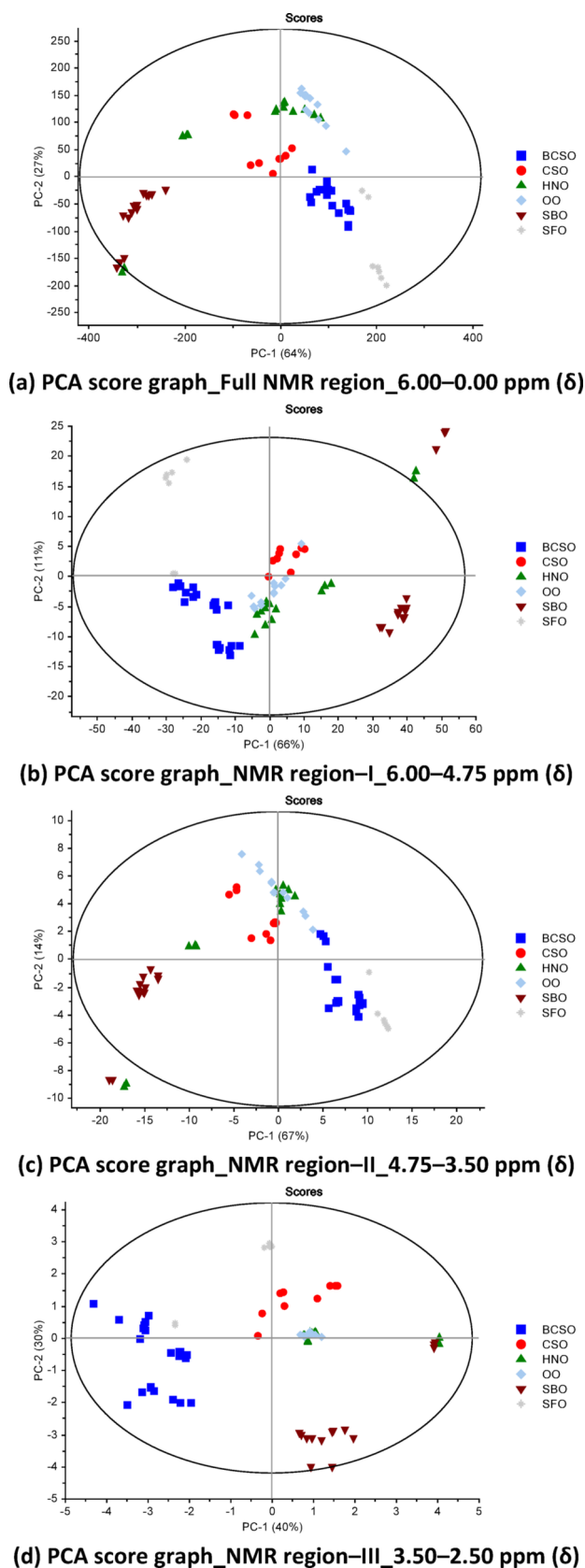


Fig. 2 Cooman's plots for the discrimination of BCSO and other edible oil samples recorded from SIMCA in the region of 6.00–0.00 δ

the *bis*-allylic protons of the $-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-$ groups (*linoleic group*, *USFAs*). The signals **F**, **E** and **D** (region-IV) at low frequencies (2.25 ppm, 2.14 ppm and 1.93 ppm, respectively) indicate the methylene protons of the $-\text{CH}_2-\text{COO}-$ (*all FAs acyl chains*) and $-\text{CH}_2-\text{CH}=\text{CH}-$ (*all USFAs*) groups. The peak **C** at 1.20 ppm (region-V) attributes the methylene protons on saturated carbon atoms [$-(\text{CH}_2)_n-$ groups *all FAs acyl chains*]. Finally, the peaks **B** and **A** at 0.89 ppm and 0.82 ppm (region-VI) represent the methyl protons of $-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_3$ (*all PUFAs acyl chains*) and $-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$ (*all FA acyl chains*) groups, respectively.

The assignment of the above-mentioned peaks could help us to know the differences of FAs types in edible oils; on the other hand, the potential of the low field ^1H -NMR spectroscopy application is valuable for the fraud of edible oils. However, the obtained spectra encourage the significant measurement bias owing to their overlapping signals and poor spectral resolutions. The spectra of all samples are too similar (Supplementary Fig. 1a), and it was very hard to differentiate them. In these spectra, 32 different signals were used for the authentication of samples; moreover, the proton associated these resonances showed the total relaxation during the measurements, guaranteeing qualitative and quantitative results. The identification of these signals has been performed by taking the earlier reported studies as a reference [23–28, 30–33].

Before the multivariate data analyses, the integrated ^1H -NMR data were pretreated (Supplementary Fig. 1b, c). Furthermore, the GC-FID analyses were performed to determine the FA profiles of the oil samples (Supplementary Tables 2, 3), and the content of FAs were highly accordance with the results stated in earlier literatures and Codex Alimentarius [43, 44, 46, 47].

Authentication of BCSO and other edible oils by ^1H -NMR data

The above-mentioned proton signals were employed to develop the spectral fingerprints for each type of samples. The chemical fingerprints were acquired for the 34 different unadulterated oil samples by integrating the signals obtained from the spectra of full (6.00–0.00 δ) and selected regions (*region-I to -VI*). Afterwards, the multivariate qualitative data analyses including PCA (Fig. 2 and Supplementary Fig. 2), SIMCA (Fig. 3 and Supplementary Figs. 3, 4, 5, 6, 7, 8), HCA (Fig. 4 and Supplementary Figs. 9, 10, 11, 12, 13, 14) and LDA (Fig. 5 and Supplementary Table 1) were constructed.

Figure 2 Score plots for the BCSO and other edible oil samples recorded from PCA in the regions of a 6.00–0.00 δ ,

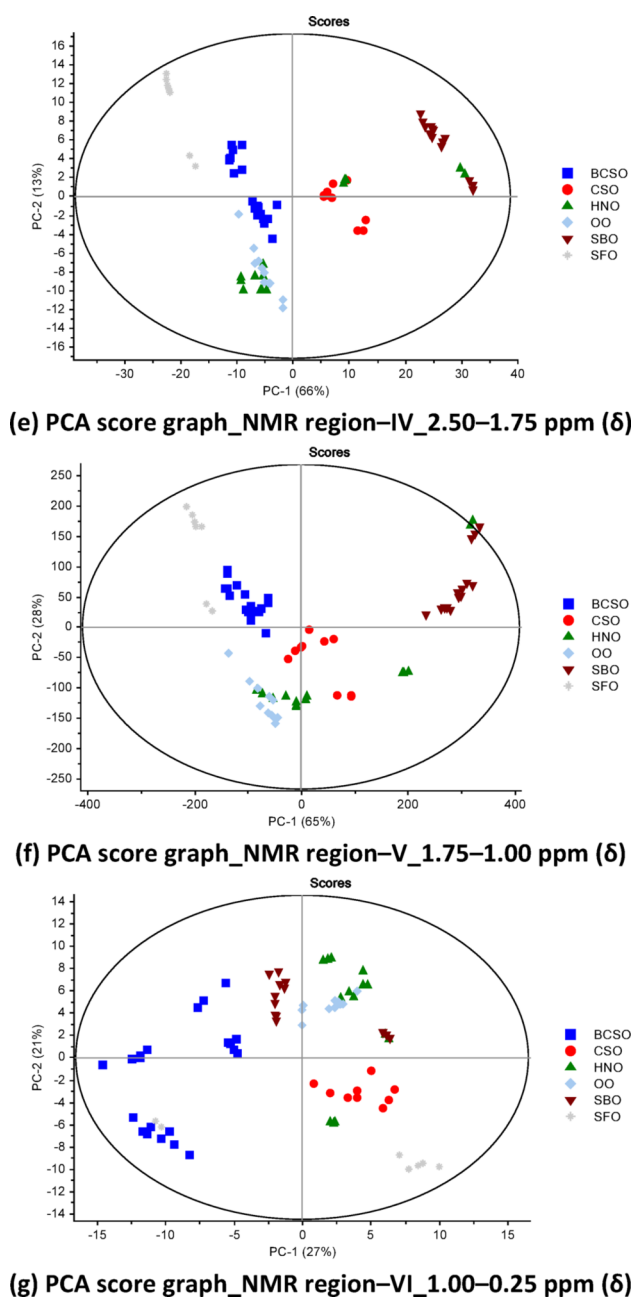


Fig. 2 (continued)

b 6.00–4.75 δ, **c** 4.75–3.50 δ, **d** 3.50–2.50 δ, **e** 2.50–1.75 δ, **f** 1.75–1.00 δ, and **g** 1.00–0.25 δ.

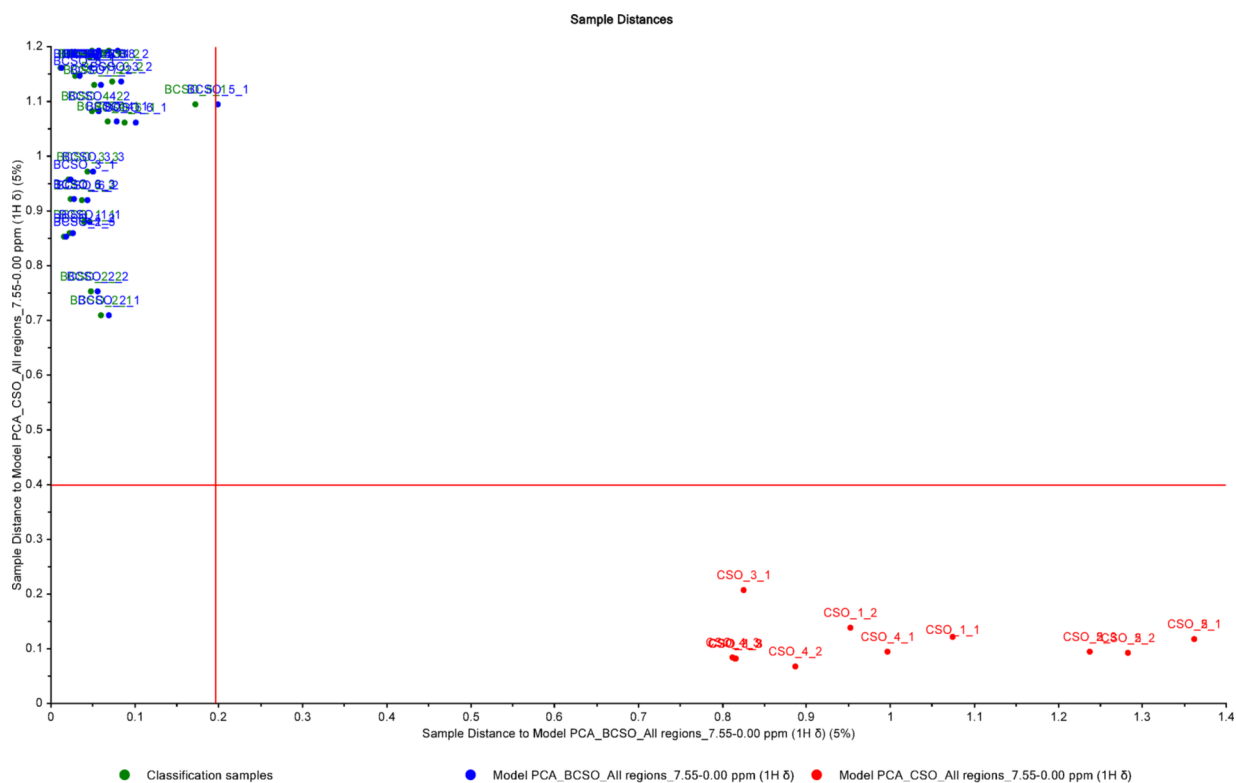
For the full NMR region, a matrix [$D_{8089 \times 102}$; 8089 rows symbolize to the full ^1H -NMR range (6.00–0.00 δ), the 102 columns symbolize to the diverse kinds of oils with their 3 repeat analyses] was constructed, and then it was imported to the Unscrambler[®] X10.4 software for the chemometrics evaluation. The integrated signals were also grouped into the six main spectral regions (Table 1) based on the chemical shifts that symbolize the protons of saturated and unsaturated FAs in triglycerides. For these spectral regions, different

matrices were then constructed [$D_{1012 \times 102}$ (region-I; 6.00–4.75 δ); $D_{1011 \times 102}$ (region-II; 4.75–3.50 δ); $D_{809 \times 102}$ (region-III; 3.50–2.50 δ); $D_{606 \times 102}$ (region-IV; 2.50–1.75 δ); $D_{607 \times 102}$ (region-V; 1.75–1.00 δ) and $D_{606 \times 102}$ (region-VI; 1.00–0.25 δ)], and they were consequently transferred to the Unscrambler[®] X10.4 software.

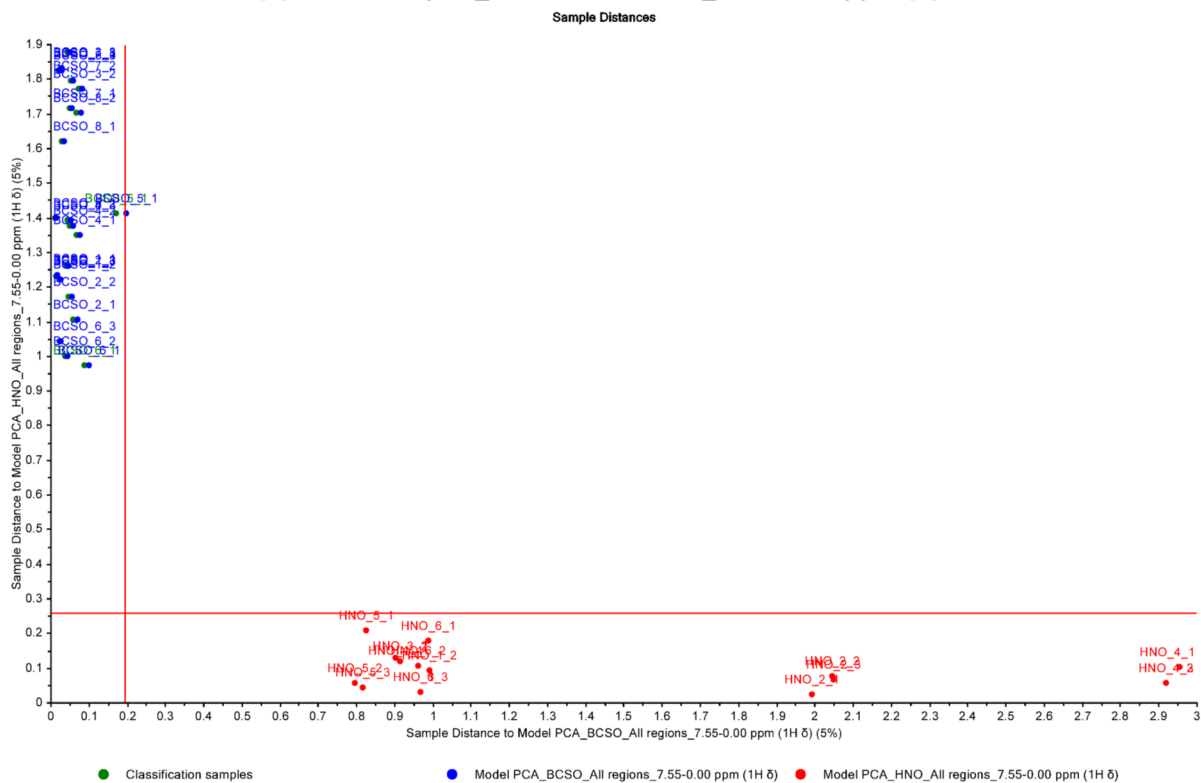
In the first stage of multivariate data analyses, the spectral data in the matrices were analyzed by the PCA to investigate the success of the proposed approach, as well as to classify the BCSOs and other edible oils according to their origins. The PCA score graphs were separately constructed for the oil samples in different origins (BCSOs, CSOs, SFOs, HNOs, VOOs and SBOs) (Fig. 2a and b–g) with a non-linear iterative partials least squares (NIPALS) method. As clearly seen in Fig. 2, the PCA plots based on the low-field ^1H -NMR data demonstrate that the tested oils could be successfully classified according to their origins. In the Fig. 2a, the PC1 signifies 64% of the variability; on this factor, the samples of HNOs and OOs appear to be too close to each other (except the suspected HNO samples), it has a positive score, and the samples were well classified based on their origins. On the same PC, the cold pressed BCSOs and refined SFOs (except a suspected SFO sample) have positive scores and well separated from each other, and they are discriminated from the other oil samples. The refined SBO samples have negative scores and are well-discriminated from all samples, while the refined CSO samples are in the middle close to negative scores. The PC2 symbolizes 27% of variability, and it classifies the HNOs, OOs and CSOs by positive scores from the samples of BCSOs, SFOs and SBOs by negative scores.

On the other hand, all suspected samples under study were successfully identified with their sample codes by using the score graphs built within the full NMR region (Supplementary Fig. 2). The findings of the PCA score plots built with the data of 6 different spectral regions are also summarized in Fig. 2b–g. All of these score plots have satisfactory eigen values that could be successfully employed

to classify samples to their origins. In the same graphs, the cold pressed BCSO samples almost classified from others. The PCA graphs constructed from the data of full (6.00–0.00 δ; correspond to the glycerol backbones in triglyceride molecules) and region-VI (1.00–0.25 δ; correspond to the methylene protons on all fatty acid acyl chains) offer excellent predictions for the classification of BCSOs and different types of oils (Fig. 2a–g); on the other hand, the HNO and OO samples were inadequately differentiated from each other (Fig. 2g). The obtained results were quite consistent



(a) Cooman's plot_BCSOs from CSOs_6.00–0.00 ppm (δ)

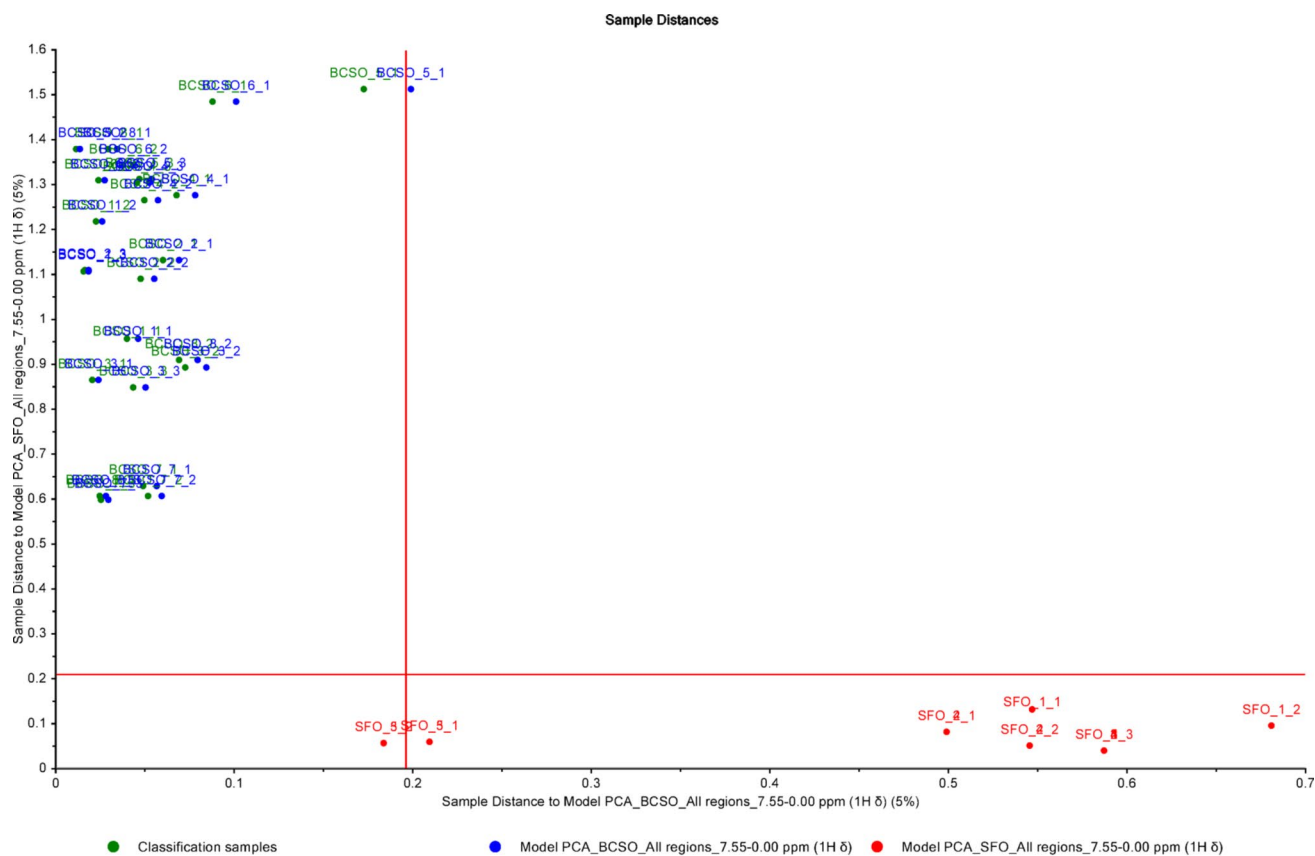


(b) Cooman's plot_BCSOs from HNOs_6.00–0.00 ppm (δ)

Fig. 3 Cooman's plots for the discrimination of BCSO and other edible oil samples recorded from SIMCA in the region of 6.00–0.00 δ



Fig. 3 (continued)



(e) Cooman's plot_BCSOs from SFOs_6.00–0.00 ppm (δ)

Fig. 3 (continued)

with the data of GC–FID analyses under study (Supplementary Tables 2, 3).

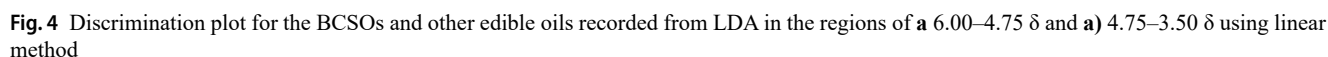
Next, the supervised classification model SIMCA made of two groups (*that is, the training groups/samples wherein the separate PCA models*) were constructed within the data of full (Fig. 3) and selected NMR regions (*region-I to -VI*) (Supplementary Figs. 3, 4, 5, 6, 7, 8). This supervised model, SIMCA, is constructed by calculating two independent PCAs and graphing the residual distances of samples of each groups one vs. the other in Cooman's plot [46]. The Cooman's plots under study were utilized to assess the authentication where is owned by a definite group should locate within the classification limits (*95% confidence limits for critical distance*). As obviously seen in Fig. 3, the BCSO and others were all classed within two different groups with no-misclassification, proving the perfect predictability, and the obtained results were comparable to the earlier studies [4, 28, 30, 32, 42–44, 46].

The Cooman's plots also demonstrated a good specificity as other samples were not located into either class. All plots in Fig. 3 and Supplementary Figs. 3, 4, 5, 6, 7, 8 provided satisfactory distances, and in these graphs all variables demonstrated high discrimination and modeling powers, which

revealed the discrimination ability of constructed models. The BCSOs and other edible oils were well discriminated into two groups with no-misclassifications by SIMCA build over the spectral data of 6.00–4.75 δ , 4.75–3.50 δ and 2.50–1.75 δ regions. Hence, the SIMCA models could be fruitfully used for the discrimination of samples including different types of triglycerides, FAs, and also for the authentication of them.

Figure 3 Cooman's plots for the discrimination of BCSO and other edible oil samples recorded from SIMCA in the region of 6.00–0.00 δ .

The spectral data of the full region (6.00–0.00 δ) was then employed for the construction of supervised LDA model. The linear LDA model was generated with the six predictors (BCSOs, CSOs, HNOs, VOOs, SBOs and SFOs) (Fig. 4 and Supplementary Table 4) and selected eleven different chemical shift points (2.38 δ , 2.26 δ , 2.13 δ , 2.02 δ , 1.98 δ , 1.65 δ , 1.55 δ , 1.22 δ , 0.87 δ , 0.84 δ and 0.71 δ). As clearly seen from the linear LDA score plot, a good finding was obtained for the discrimination of BCSOs from others. All of the tested oil samples were acceptably discriminated on the basis of their origins; that is, there is no misclassification of the studied samples by LDA. Consequently, the low-field



To validate the linear LDA model, the data of the testing samples were utilized and the prediction data were given by the confusion matrix, confirming the high prediction ability of proposed model and the obtained findings comparable to previous studies [28, 32, 46]. One sample of SFO (*SFO-5*) and two samples of HNOs (*HNO-2 and HNO-4*) were detected as suspected samples based on the SIMCA and PCA results, and that's why, they were described as "testing set". As clearly seen in the classification table of LDA, the HNO-2 and HNO-4 samples were located in the SBO group, while the SFO-5 was in the SFO group (Supplementary Table 4). Therefore, these results reveal that the low-field ^1H -NMR technique has a great potential for the discrimination of edible oil samples based on their origins.

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Table 2 The results of PLS-R analysis for the adulterated BCSOs recorded from the region of 6.00–0.00 Δ

PLS-R analysis results for the adulterated BCSOs with CSOs or SFOs recorded from the region of 6.00–0.00 δ									
Sample number		Calibration				Validation			
		Slope	Offset	RMSEC	R ²	Slope	Offset	RMSECV	R ²
<i>CSO-1</i> (%0–%50)									
BCSO-1	9	0.9988	0.0212	0.5815	0.9988	0.9987	0.0215	0.6245	0.9986
BCSO-2	9	0.9978	0.0379	0.7778	0.9978	0.9963	0.0511	0.8644	0.9973
BCSO-3	9	0.9993	0.0113	0.4240	0.9993	0.9992	0.0081	0.4592	0.9992
BCSO-4	9	0.9995	0.0093	0.3848	0.9995	0.9993	0.0080	0.4165	0.9994
<i>CSO-2</i> (%0–%50)									
BCSO-1	9	0.9926	0.1280	1.4294	0.9926	0.9923	0.1299	1.5346	0.9915
BCSO-2	9	0.9999	0.0004	0.0273	0.9999	0.9999	0.0014	0.0309	0.9999
BCSO-3	9	0.9993	0.0113	0.4240	0.9993	0.9992	0.0081	0.4592	0.9992
BCSO-4	9	0.9995	0.0093	0.3848	0.9995	0.9993	0.0080	0.4165	0.9994
<i>SFO-1</i> (%0–%50)									
BCSO-1	9	0.9990	0.0178	0.5335	0.9990	0.9990	0.0224	0.5626	0.9989
BCSO-2	9	0.9963	0.0634	1.0063	0.9963	0.9948	0.0814	1.0812	0.9958
BCSO-3	9	0.9982	0.0306	0.6992	0.9982	0.9968	0.0431	0.7757	0.9978
BCSO-4	9	0.9991	0.0158	0.5029	0.9991	0.9990	0.0167	0.5389	0.9989
<i>SFO-2</i> (%0–%50)									
BCSO-1	9	0.9991	0.0177	0.5335	0.9989	0.9990	0.0223	0.5625	0.9988
BCSO-2	9	0.9896	0.1794	1.6925	0.9896	0.9884	0.1925	1.7945	0.9883
BCSO-3	9	0.9999	0.0011	0.1299	0.9999	0.9999	0.0005	0.1582	0.9999
BCSO-4	9	0.9943	0.0993	1.2593	0.9943	0.9938	0.1026	1.3495	0.9934

CSO refined cotton seed oil, SFO refined sunflower oil, BCSO black cumin seed oil, R² R-squared, RMSECV root mean square error of cross validation, RMSEC root mean square error of calibration

Quantification analysis of adulterated cold pressed BCSO by ¹H-NMR data

The ¹H-NMR spectra were recorded for the adulterated BCSO samples ($n = 144$) [cold pressed BCSOs adulterated with refined CSO (1–50% v/v, $n = 72$) or SFO (1–50% v/v, $n = 72$)]. In order to detect classification limits between the unadulterated and adulterated BCSO samples, the qualitative SIMCA and quantitative PLS-R graphs were built over the spectral data of 6.00–0.00 δ region. The unadulterated cold pressed BCSOs and adulterated BCSOs were evaluated into two different groups by the SIMCA (Supplementary Figs. 16, 17, 18, 19, 20, 21, 22, 23). The satisfactory classifications between the unadulterated BCSO-2 and its adulterated samples with SFO-1 or CSO-1, as well as between the unadulterated BCSO-4 and its adulterated samples with CSO-1 or CSO-2, were achieved. Their SIMCA models classified 100% of these samples correctly consistent with its cluster, namely samples were incorrectly grouped into their own clusters, proving the similarities in fatty acid contents between these samples (see Supplementary Tables 2, 3). Moreover, the detected classification limit for the adulterants in the cold pressed BCSOs were <1%, which is lesser than the authentication limits said by the previous studies [48, 49].

The supervised PLS-R analyses were performed over the data of 6.00–0.00 δ region for each adulterated set. To

probe the accuracy of developed PLS-R models, a cross-validation method was applied for each calibration graph (Table 2). According to the cross-validation method, thirty-six samples were utilized as “validation set” (25% of samples) and one hundred eight samples as “calibration set” (75% of samples). The regression results [slope, offset, RMSEC, RMSECV, and R-squared values] are given ($n = 16$ different sets for the mixtures of BCSO-CSO and BCSO-SFO) in Table 2 and Supplementary Figs. 24, 25, 26, 27. As depicted in Table 2, good linearity results were obtained for the calibration plots. The limits of detection (LOD, namely RMSEC) for adulterants in BCSOs were 0.03% for the BCSO-CSO sets with R² of 0.9999% and the RMSEC was 0.13% for the BCSO-SFO sets with R² of 0.9999%, respectively, which are highly lesser than the adulteration limits reported by the earlier studies [28, 30, 32, 33]. Thus, the satisfactory calibration results were obtained for the detection of cold pressed BCSOs adulteration, and this regression method might be highly valuable as no knowledge of adulterant is necessary, and it could be successfully used to quantify the content of adulterants.

Conclusions

In conclusion, the 60 MHz ^1H -NMR spectroscopy methods combined with chemometrics revealed a good potential for the recognition of cold pressed BCSO adulteration. The matrices constructed from the ^1H -NMR data were employed for the detection of BCSOs adulteration by employing the qualitative (*PCA*, *HCA*, *SIMCA* and *LDA*) and quantitative (*PLS-R*) multivariate data analyses. A total of 100% of samples were acceptably discriminated according to their own origins by linear *LDA* model; that is, no misclassification of the tested samples. In particular, the quantitative recognition of the presence of refined CSOs and SFOs might be performed from the lowest adulterant amounts of 0.03% (w/w) and 0.13% (w/w), respectively. Hence, the research highlights that the low-field benchtop ^1H -NMR spectroscopy is an excellent methodology to reveal the recognition of BCSOs adulteration with refined edible oils. The simplicity and applicability of this approach could be also utilized in the authentication of the most popular food frauds, and these findings might open new opportunities for the further adulteration analysis based on low field NMR strategy.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest Fatma Nur Arslan declare no competing interests. Suleyman Dincer declare no competing interests.

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