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Studies on Detecting Olive Oil Adulteration by Spectroscopic Techniques

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ABSTRACT

This study focused on the application of ^{1}H NMR spectroscopy to identify adulteration of two olive oil varieties (Koroneiki and Coratina) with cheaper vegetable oils (sunflower, soybean and corn oils).General assignments of ^{1}H NMR spectra of olive oils and their binary admixtures with 10% (w/w) (sunflower, soybean and corn oils) werediscussed. The most obvious difference between the spectra of olive oils and their binary admixtures spectra is the appearance of the signal around 2.04 ppmdue to allylic protons of linoleic acid in the binary admixtures while being absent in pure olive oils.This peak could be functioned as indicator of adulteration of olive oil with high linoleic acid oils. Furthermore, the peak integrals at 2.7ppm showed a good correlation with the total sum of linoleic and linolenic acids.It was found from fatty acids profile that,adulteration of extra virgin olive oils with high linoleic acid oils could be detected with 10% (w/w) addition of both soybean and corn oils while, sunflower oil addition could not be found out at 10% (w/w).

Keywords*:Adulteration, Fatty acids, ^{1}H Nuclear magnetic resonance ,Olive oil, Spectroscopic techniques*.

1. INTRODUCTION

Olive oil, the oil obtained solely from the fruit of the olive tree (*Oleaeuropaea L.*), is now becoming popular throughout Mediterranean and non-Mediterranean areas[1]. The consumption of olive oil has increased recently due to its excellent organoleptic, nutritive properties and health benefits, namely the reduction of risk factors and prevention of the occurrences of chronic diseases like diabetes and obesity, together with the current tendency of consumers to select minimally processed foods, have prompted a re-assessment of its consumption in a regular diet [2-5].

Olive oil is frequently subjected to be adulterated with other edible oils of lower commercialvalue [6].The detection of adulteration in EVOOs is a particular concern inthe food industry. The adulteration of extra virgin olive oil withother cheaper oils can lead to significant profits for the unscrupulousvendor or raw material supplier. Therefore, continuouscaution is required to control the adulteration of EVOOs. Although the

adulteration is done by economic reasons, the action can affect the quality of food, where olive oil is a component [7].

Several researchers have developed various analytical methods to detect the adulteration resulting from the blending of EVOO with other vegetable oils. Such methods are liquid chromatography for analysis of the triglyceride compositions [8], electronic nose for measurement of the aroma and/or volatile compounds andgas chromatography for determination of fatty acid and sterol contents[9]. The official bodies have established limits with regard to the content of fatty acids in olive oil and these limits are used for the discrimination between genuine olive oil and other vegetable oils.

Also fingerprinting techniques such as NMR [10, 11], MIR [12], NIR [13], FT-IR, FT-MIR,and FT-Raman [4, 14-16] spectroscopies,MS [17], GC_GC-TOF-MS [18-20], and DNA fingerprinting[21, 22] have been used for the determination of foodauthenticity.

NMR spectroscopy, one of the most promising spectroscopic techniques for the analysis of complex systems, such as food matrices, has extensively been used for oil analysis and has been established as a valuable tool for the assessment of the quality and authenticity of olive oil. ^{1}H and ^{13}C NMR spectroscopy shows a number of advantages relative to other analytical techniques; it is fast (less than 5min are required to record an one-dimensional ^{1}H NMR spectrum of an olive oil sample in $CDCl_3$); it needs no calibration with internal standards or separation of the various components prior to the analysis; it shows remarkable selectivity, in as much it identifies unknown compounds at a molecular level; it gives a wealth of information in a single experiment; it is quantitative with excellent repeatability and reproducibility.Since 1993, NMR has become an AOCS Official Method to determine solid fat contents (SFC) of fats and oils in the food industry, particularly in the bakery, confectionery and margarine industries. Although several analytical methods already exist for the detection of virgin olive oil (VOO) adulteration, NMR fingerprinting was proven to be a much more effective method in the authentication of VOOs based on their geographical origin [23-25].

In this study, two olive oil varieties, Koroneiki and Coratina samples, pure and combined into binaryblends withcheaper vegetable oils (sunflower, soybean and corn oils) have been analyzed by gas chromatographyand ^{1}H NMR spectroscopy in orderto discriminate between them, to determine their lipid compositionand to detect the adulteration of extra virgin olive oils.

2. MATERIALS AND METHODS

2.1 MATERIALS

Olive samples were collected from Giza governorate in Egypt. Koroneiki and Croatina are two different species of olive utilized in this work. The olive fruits were immediately transported on the same day, to the laboratory where they were transformed into oil within 24 hours and kept in brown glass bottles at -5 ºC till time of analysis. Sunflower, soybean and corn oils were obtained from Arma Oil Industries (Tenth of Ramadan City, Sharqia). Binary admixtures of olive oils (Koroneiki and Croatina varieties) and 10% (w/w) of each of sunflower, soybean and corn oil were prepared.

2.2 METHODS

2.2.1 FATTY ACID COMPOSITION BY GC

The fatty acid composition of the acylglycerols as theirmethyl esters was prepared according to ISO 12966-2[26].The fatty acid methyl esters (FAME) were analyzed by an Agilent 6890 series gas chromatograph equipped with a DB-23(60 m X 0.32 mm X0.25µm) capillary column (Agilent Technologies Inc., CA, USA). One micro liter of FAME mixture was injected into the GC system with split/splitless injector and flame ionization detector (FID). The inlet temperature was 250 °C and the split ratio was 50:1. The carrier gas was hydrogen at 1.6 ml/min constant flow. The oven temperature was programmed at initial 150 °C, held for 1 min, followed by increase of 10 °C/min up to 170 °C, held for 5 min, followed by increase to 220 °C during 10min. holding for 3 min. The detector was set at 270 °C with 450 ml/min airflow, 40 ml/min hydrogen flow, and 25 ml/min nitrogen makeup flow. Fatty acid methyl ester standards were used to identify the peaks.

2.2.2 ^{1}H-NMR SPECTRAL ANALYSIS

^{1}H-NMR spectral analysis was carried as follows[27]. A number of 6 sets of extra virgin olive oil (Koroneiki and Croatina varieties) mixed with each of the adulterant oils; sunflower, corn and soybean oils in mixing ration of 10% on (w/w) basis without sample preparation procedures, plus 2 sets of pure oils; extra virgin olive (Koroneiki and Croatina varieties) were dissolved in deuterated chloroform ($CDCl_3$). ^{1}H-NMR spectra were recorded on a Varian Mercury300 BB (NMR300) spectrometer at NMR lab, Faculty of science, Cairo University, Egypt. Observing ^{1}H at 300.06 MHz at temperature of 30^{o}C, ^{1}H-NMR spectra were acquired using spectral width of 6600.7 Hz, relaxation delay of 1 sec, 5 repetitions, acquisition time of 4.004 sec. and with a total time of 36 min, 56 sec. Chemical shifts were expressed in δ units (ppm).

3. RESULTS AND DISCUSSION

3.1 FATTY ACID COMPOSITION BY GC

Gas chromatography (GC) with flame-ionization detection is undoubtedly the technique that would be chosen in most circumstances for determination of fatty acid compositions, after conversion to simple ester derivatives. Analysis of fatty acids compositions seems to be a very useful technique for monitoring the authenticity of high price EVOO. The main fatty acid in EVOO is oleic ($C_{18:1}$) that account for about 2/3 of the total fatty acids percentage. Palmitic ($C_{16:0}$) and linoleic ($C_{18:2}$) acids, each of them, only represent not more than 20% of the total fatty acids composition. Meanwhile, sunflower, soybean and corn oils have linoleic as the predominant fatty acid followed by oleic acid and Palmitic acids.This difference can be used to analyze EVOO adulterated with high linoleic acid oils. It is found that, with the addition of 10%(w/w) (sunflower, soybean and corn oils) to the extra virgin olive oil the four fatty acids (palmitic, oleic, stearic and linoleic acid) changed and their limits being within those for extra virgin olive oil i.e. gives no indication of adulteration. On the other hand, linolenic acid $C_{18:3}$ increased with the addition of sunflower, soybean and corn oils and the significant change was noticed in addition of soybean oil. In Table 1 Coratina variety, the linolenic percentage was out of extra virgin olive oil

limits, ≤ 1, with the 10% addition of soybean oil since its value was 1.38%, while the linolenic percentage for Koroneiki olive oil admixture was 1.32%, consecutively.

Corn oil has much more pronounced characteristic of transfatty acid content that its value in the binary admixturesexceeded the limits of edible olive oil since its values 0.13 for Coratina variety and 0.09 for Koroneiki variety and the limit established by IOC [28] and CODEX STAN[29] were transfatty acid content (% transfatty acids) for edible virgin olive oils: $C_{18:1}$ T% ≤ 0. 05 and $C_{18:2}$ T + $C_{18:3}$ T % ≤ 0.05.

From fatty acids profile of the binary admixture of extra virgin olive oils with the high linoleic oils it could be concluded that, the addition of both soybean and corn oil may be detected with 10% (w/w) adulteration percentage. On the other hand, sunflower addition to extra virgin olive oil could not be found out at 10% (w/w) addition. This results are in agreement with those of Škevin et al. [30] who found that fatty acid composition enabled the identification of addition of 20 % of sunflower oil to extra virgin olive oil and Jabeur et al. [31] who stated that the adulteration could also be detected by the increase of the trans fatty acid contents with 10% pomace olive, 3 %soybean, 3% sunflower, 2 % corn, and 10 % palm oils.

Table 1: Fatty acids profile of koroneiki (k)and coratina(C) olive oils and their binary admixtures*.

Carbon number	K	KSO	KS	KC	C	CSO	CS	CC
C16:0	14.39	13.68	13.99	13.98	14.49	13.69	14.29	14.15
C16:1	0.85	0.78	0.85	0.84	0.89	0.89	0.88	0.81
C17:0	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
C17:1	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
C18:0	2.54	2.64	2.74	2.49	2.37	2.54	2.57	2.34
C18:1	71.05	66.36	66.35	66.73	70.46	65.69	65.62	65.99
C18:2 trans	-	-	-	0.09	-	-	-	0.13
C18:2	9.21	14.69	13.65	14.00	9.78	15.19	14.13	14.59
C18:3	0.88	0.81	1.32	0.86	0.94	0.87	1.38	0.91
C20:0	0.48	0.45	0.47	0.46	0.45	0.44	0.44	0.44
C20:1	0.36	0.32	0.36	0.37	0.39	0.39	0.41	0.39
C22:0	0.12	0.17	0.15	0.14	0.10	0.18	0.14	0.12

*Binary admixtures with 10%(w/w) Sunflower oil (KSO, CSO), 10%(w/w) Soybean oil (KS, CS) and 10%(w/w) corn oil (KC, CC) for koroneiki and coratina olive oils respectively.

3.2 ^{1}H-NMR SPECTRAL ANALYSIS

The choice of this added percentage and not less than 10% w/w was according to Guillèn and Ruiz[28] who stated that mixtures of hazelnut or of sunflower oils with olive oil can be detected by this method whenever the proportion of hazelnut is higher than 25% and that of sunflower higher than 10%.Vegetable oils contain various proportions of saturated, oleic, and polyunsaturated acyl groups and the resulting signals have different chemical shifts and shapes depending on these proportions. A careful observation of the shape and number of peaks of each signal present in the spectra allows finding significant differences between vegetable oils of different composition.

The general assignment of the signals of Koroneiki and Coratina olive oils and their binary admixtures with 10% (w/w) corn, 10% (w/w) sunflower and 10% (w/w) soybean oils spectra to the different kind of the proton resonances of the triacylglycerol acyl chains (the structures of the major fatty acids of olive oil and the basic structure of triacylglycerols) are given inTable (2).Signal (1) around 0.85, ppm, is produced by the overlapping of the triplet signals (t) of methyl group hydrogen atoms, the terminal methyl protons, of saturated, oleic (*n*-9) and linoleic (*n*-6) acyl groups and signal (2) at 0.95, ppm, is a triplet due to the methyl hydrogen atoms of *n* -3 acyl groups. The difference in chemical shifts between both methyl hydrogen atom signals is due to their proximity to the double bond of the chain. Signal (3) at 1.28 ppm, is due to the hydrogen atoms of those methylene groups either in position β or further from olefinic groups, or in position γ or further from carbonylic groups inside the triglyceride molecule. Signals (4) around 1.58 ppm and (7) at about 2.28 ppm, are due to methylene hydrogen atoms in β and α positions, in relation to the carbonyl group, respectively. Signal (5), around 1.9 ppm, is due to α- methylene hydrogen atoms in relation to a single double bond, also named allylic hydrogen atoms, and signal (8) at 2.7 ppm arouse from the overlapping of the signals of α methylene hydrogen atoms in relation to 2 double bonds, also named *bis*-allylic hydrogen atoms. Signal (9) resonate at 4.09 and 4.3 ppm (dd) as a result of the hydrogen atoms on 1 and 3 carbon atoms of the glyceryl group and signal (10) around 5.25 ppm and not baseline separated is due to the hydrogen atom on the carbon atom 2 of the glyceryl group.

This latter signal overlaps slightly with signal (11) a double bond –CH=CH– in the acyl chain contributes an 'olefinic' peak at 5.3 ppm due to olefinic hydrogen atoms of the different acyl groups.

Table 2:Assignment of the main resonances in the ^{1}H NMR spectra ofkoroneiki (k)and coratina(C) olive oils and their binary admixtures*

Peak no.	Chemical shift (ppm)								Functional group	Attribution
	K	KC	KSO	KS	C	CC	CSO	CS		
1	0.85	0.84	0.86	0.86	0.85	0.87	0.86	0.86	-C**H**$_3$	All acids except linolenyl

2	0.95	0.91	0.93	0.95	0.94	0.96	0.92	0.96	-C**H**$_3$	linolenyl acyl chains
3	1.28	1.27	1.29	1.29	1.28	1.29	1.28	1.29	-(C**H**$_2$)n	All acyl chains
4	1.58	1.57	1.59	1.60	1.59	1.60	1.59	1.59	-C**H**$_2$-CH_2COOH	All acyl chains
5	1.99	1.98	2.00	2.00	1.99	2.01	2.04	2.00	-C**H**$_2$-CH= CH	All unsaturated fatty acids
6		2.02	2.04	2.04		2.05	2.04	2.04	-C**H**$_2$-CH= CH	Linoleyl acyl chains
7	2.28	2.26	2.28	2.29	2.28	2.29	2.28	2.29	-C**H**$_2$-COOH	All acyl chains
8	2.74	2.73	2.75	2.75	2.74	2.76	2.74	2.75	-CH=CH-C**H**$_2$-CH=CH	Linoleyl and linolenyl
9	4.08-4.30	4.07-4.29	4.09-4.31	4.09-4.31	4.08-4.30	4.09-4.31	4.09-4.31	4.09-4.31	-C**H**$_2$-OCOR	Glycerol (triacylglycerols)
10	5.24	5.24	5.26	5.26	5.24	5.27	5.26	5.26	-C**H**-OCO	Glycerol (triacylglycerols)
11	5.31	5.31	5.32	5.32	5.31	5.32	5.31	5.32	-C**H** =CH-	All unsaturated fatty acids

* Binary admixtures with 10%(w/w) Sunflower oil (KSO, CSO), 10%(w/w) Soybean oil (KS, CS) and 10%(w/w) corn oil (KC, CC) for koroneiki and coratina olive oils respectively.

The most conspicuous difference between the spectra of olive oils and their binary admixtures spectra is the appearance of the signal (6) around 2.04, absent in pure olive oil, due to allylic protons of linoleic acid. This peak is a characteristic only of adulterated olive oils for both Koroneiki and Coratina olive oils Fig(1) that could be functioned as indicator of adulteration of olive oil. By a careful study of the fatty acids profile of olive oils and their binary admixtures it could be noticed that linoleic acid percent did not exceed the limit established for extra virgin olive oil (max.15.19%), however the appearance of this peak gave a good indicator of the presence of foreign oils, especially high linoleic acid oils, in olive oil.

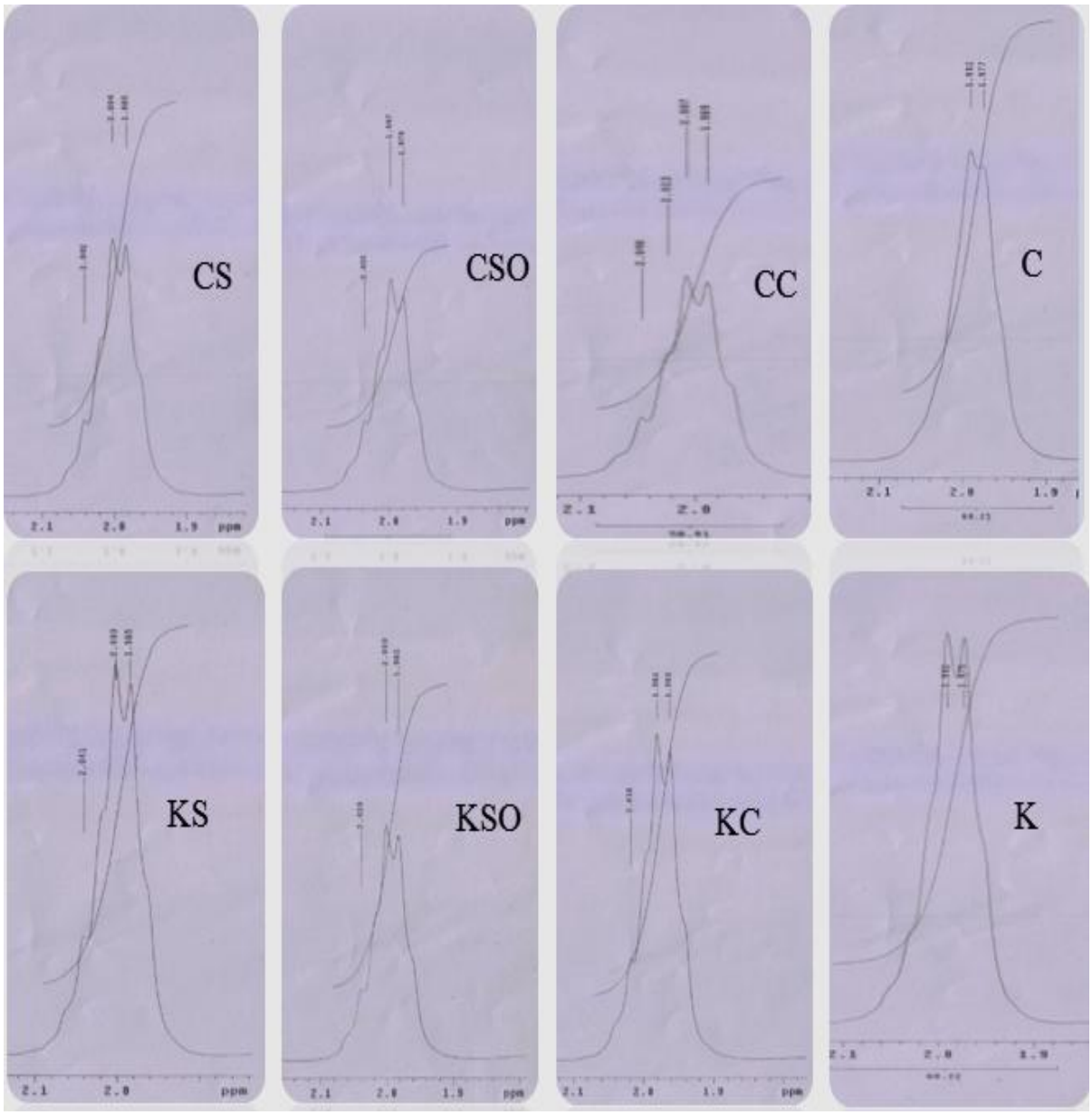

Figure 1: assignments of the resonances around 2.04 ppm in the ^{1}H NMR spectra of olive oils and their binary admixtures*

* Binary admixtures with 10%(w/w) Sunflower oil (KSO, CSO), 10%(w/w) Soybean oil (KS, CS) and 10%(w/w) corn oil (KC, CC) for koroneiki and coratina olive oils respectively.

The appearance of the spectra of all studied samples is very similar, with differences only apparent in the intensities of the peaks, although a careful observation of the extended spectra shows significant differences between them.The integrals of the ^{1}H NMR signals are strictly proportional to the number of hydrogen atoms present in each functional group and the number of functional groups present in olive oil and are basically due to the hydrogen atoms of the main components, *i.e.* of the triacylglycerols. The ^{1}H NMR resonance groups were

integrated and their percentage from the total signal was estimated so as to evaluate the composition of the oils and to differentiate between them, offering a direct route to quantitative analysis. Table 3 represents the integrations of signals of the spectra of Koroneiki and Coratina olive oil and its binary admixtures with 10% (w/w) corn, 10% (w/w) sunflower and10% (w/w) soybean oils.

The largest contribution to the total signal obtained for an oil sample is given by the 1.28 ppm signal with about 58% representing the methylene groups $(CH_2)_n$of all the fatty acids, followed by 2 ppm, with contributions of 10%, 0.85 ppm, with contributions of about 8% and signals at 5.3 ppm showing percentage of 7% for olive oils and their binary admixtures. The lowest signal was given by the 0.95 ppm group (terminal methyl group of linolenic acid) with integrals of about 0.48%. The signal of methyl group of linolenic acid in olive oil is quite small, so the more suitable quantification method is based on the comparison of signal (2) and the nearby ^{13}C satellites of signal (1), whose amount is exactly 0.57% of the intensity of signal(1) [32].

Since all the signals are correlated, high 0.87 ppm signals and very low 0.95 ppm signals indicate that Koroneiki and Coratina olive oils and their binary admixtures with 10% (w/w) corn, 10% (w/w) sunflower and 10% (w/w) soybean oils have lower contents of linolenic acid. In addition, higher signals 2, 2.73 and 5.3 ppm for all samples, show a higher degree of unsaturation. The integral of peak resonating at 2.73 ppm was chosen as this reflects the amount of polyunsaturated fatty acids, linoleic and linolenic acids that its value showed a good correlation with the fatty acids profile of olive oils and their blends, increases with increasing the ratio of linoleic and linolenic acids. The relationship between the total sum of linoleic and linolenic acids and their corresponding integrals at 2.7 ppm for olive oils and their binary admixtures with 10% (w/w) (corn, soybean and sunflower oils) These values were 10.09, 14.86, 14.97 and 15.5% total sum of linoleic and linolenic acids and their corresponding integrals are 0.37, 0.58, 0.57 and 0.74% at 2.7 ppm for Koroneiki olive oil and its binary admixtures with 10% (w/w) corn, sunflower and soybean oils respectively.On the other hand, Coratina olive oil and its blends exhibited analogous relationship between the integral of peak around 2.73 ppm and the amount of polyunsaturated fatty acids except for binary admixture with soybean oil which showed some deviations and their values were 10.72, 15.5,15.51 and 16.06 total sum of linoleic and linolenic acids and their integrals were 0.17, 0.54, 0.66 and 0.41% at 2.7 ppm for Coratina olive oil and its binary admixtures with 10% (w/w) (corn, sunflower and soybean oils) respectively.

Table 3:Integrals of the ^1H NMR main resonance groups forKoroneiki (K)and Coratina(C) olive oils and their binary admixtures*.

Chemical shift (ppm)	0.85	0.95	1.23	1.58	1.99	2.27	2.73	4.08-4.30	5.30
K	8.23	0.047	58.12	6.68	9.94	6.01	0.37	3.88	6.74
KC	8.42	0.048	57.65	6.64	9.86	5.95	0.58	3.86	7.01

KSO	8.58	0.049	57.93	6.43	9.96	5.79	0.57	3.73	6.98
KS	7.94	0.045	57.10	6.87	10.11	6.08	0.74	3.90	7.22
C	8.83	0.050	58.53	6.30	9.77	5.81	0.17	3.85	6.70
CC	8.52	0.049	57.70	6.52	9.85	5.89	0.54	3.90	7.06
CSO	8.21	0.047	57.13	6.77	10.12	5.98	0.66	3.87	7.24
CS	8.48	0.048	58.39	6.56	9.78	5.84	0.41	3.67	6.83

* Binary admixtures with 10%(w/w) Sunflower oil (KSO, CSO), 10%(w/w) Soybean oil (KS, CS) and 10%(w/w) corn oil (KC, CC) for koroneiki and coratina olive oils respectively.

4. CONCLUSIONS

In short, this study evidences that ^{1}H NMR spectroscopy is a very useful technique in the study of several aspects of edible oils and fats.As core conclusion we can state that NMR is faster than most ofthe techniques used in oil analysis and requires only a simplepreparation of the sample. Moreover, the composition of extra virgin olive oil and seed oils mixtures could be accurately predicted using ^{1}H NMR spectroscopy.Its usefulness has been shown in the evaluation ofimportant parameters for the food industry such asdegree ofunsaturation, proportions of the different acyl groups, authentication and quality assessment of oils, aswell as oxidative oil stability.

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