Mol2Net-04, **2018**, BIOCHEMPHYS-01 (pages 1- x, *type of paper, doi: xxx-xxxx*
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Mol2Net-04

Chemometric Characterization of Chemlali Extra-Virgin Olive Oil Adulteration Mixed with Soybean Oil, Corn Oil and Sunflower Oil

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Received: / Accepted: / Published:

Abstract:
Nowadays, the fingerprinting methodologies of olive oils are dominated. They consider the entire analytical signal, which is acquired and recorded by the analytical instrument, directly from olive oil or isoleted fraction, i,e chromatogram. The shape and intensity of the recorded signal the instrumental fingerprint from the whole olive oil adulteration. Therefore, the methodolygy is based on the chemical composition (Fatty acids and Triglycerides compositions). However, Fatty acids composition as an indicator of purity suggests that linolenic acid content could be used as a parameter for the detection of extra virgin olive oil fraud with 5% of soybean oil. The adulteration could also be detected by the increase of the trans-fatty acid contents with 3% of soybean oil, 2% of corn oil and 4% of sunflower oil. The use of the ΔECN42 proved to be effective in the Chemlali extra-virgin olive oil adulteration even at low levels: 1% of sunflower oil, 3% of soybean oil and 3% of corn oil. Therefore, compared to classical methods PCA and new approach of using LDA application could represent an alternative and innovative tool for faster and cheaper evaluation of extra-virgin olive oil adulteration.

Keywords: extra-virgin olive oil adulteration; vegetables oils; triglycerides; fatty acids; sterols; LDA; PCA.

1. Introduction

Olive cultivation is widespread throughout the Mediterranean region and is important for the rural economy, local heritage and environment. In Tunisia, the olive oil sector plays an important role in the economy, providing both employment and export revenue. However, two important varieties dominate such as Chetoui and Chemlali.[1,2] In fact, extra-virgin olive oil is usually more expensive than other vegetable oils for some reasons. It is also the oil that has not undergone any other treatment than washing, decantation, centrifugation and filtration.[3] Therefore, different methods have been developed to control the falsification of the product authenticity and quality that is being perpetrated. The determinations of fatty acids and triacyglycerols (TAGs) through chromatographic methods allow the

detection of oil adulteration and the definition of the blends composition.[4–8]

Fatty acid methyl esters (FAME), obtained by alkali/catalyzed transesterification of vegetable oils can be characterized by, gas chromatography.[9–12] The methyl esters of the fatty acids under investigation are usually the molecules used for the chemical analyses establishing the limits pertaining to the content of fatty acids in olive oil that can be used for the differentiation between genuine olive oil and other vegetable oils (soybean, sunflower and corn.)[13]

TAGs are the main component of vegetable oils as they are generally found between the 97 to 99% range of the whole oil composition. The High Performance Liquid Chromatography (HPLC) quantitative analysis of TAGs are considered as an effective method for the detection of extra-virgin olive oil adulteration.[14,15]The advantage of using TAGs profile includes the distribution of fatty acids between the different stereospecific positions on the glycerol molecule. TAGs are separated according to the equivalent carbon number (ECN) and the positions of double bond(s). Until recently, the most prominent methods to detect the adulteration of extra-virgin olive oil with other vegetable oils have been the trilinolein (LLL) content and the difference between the theoretical value of TAGs with an equivalent carbon number of 42(ECN42theoretical). An appropriate software is used to compute the ΔECN42 based on data of fatty acids composition and analytical triglyceride results (ECN42HPLC).

The utmost objective of the present research study has focused on discriminating and detection of the Chemlali extra-virgin olive oil adulteration with soybean, corn and sunflower oils. For this purpose, analyses of fatty acid and triacylglycerol profiles are performed using gas and liquid chromatography. This leads to the determination of the minimum detectable levels of vegetable oils (soybean, corn and sunflower).

2. Results and Discussion

Identification of EVOO Adulteration with Other Low Cost Oils

This research study is meant to detect adulteration of EVOO by lower cost seed oils. Consequently, various blends of EVOO and soybean, corn or sunflower oil were prepared and analyzed for fatty acid and triglyceride compositions. The adulteration percentages ranged from 1to10% in order to determine a threshold of detection.

Use of the Fatty Acid and Triglyceride Compositions for the Detection of Fraud

Fatty acid and TAG compositions of the adulterated extra-virgin olive oil mixed with 1-10% (w/w) quantities of soybean, corn and sunflower oils were summarized in Figures 1, 2.

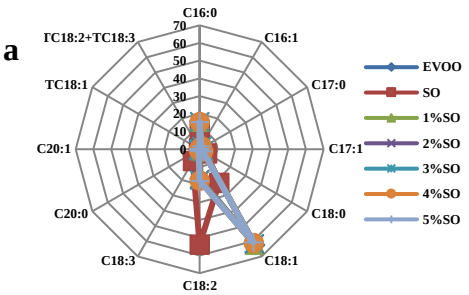
Taking into account the results presented in Figures 1, it could be concluded that the analysis of fatty acids does not produce satisfactory results with regard to the levels of adulteration investigated in this research study. The most effective parameters for the detection of adulteration are mentioned below. The linolenic acid percentage could be used as a parameter for the detection of EVOO fraud with 5% of soybean oil as well as by the increase of trans-fatty acid contents with 3% of soybean oil, 2% of corn oil and 4% of sunflower oil (Table 1 and Figure 3). None of the other fatty acids is effective for the detection of the added vegetable oil, in an extra-virgin olive oil.

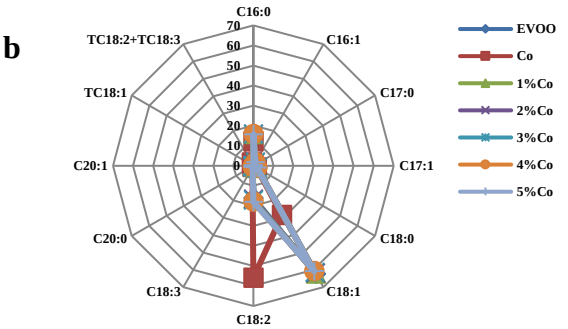
Although the composition of fatty acids in the examined seed oils is different from that of olive oils, the fatty acids experiment could not be satisfactorily used as discriminatory parameters between olive oil and the respective vegetable oil in most cases. According to Figure 2, it can be noted that the appearance of a specific triacylglycerol, normally not present in the olive oil, in oils adulterated with seed oils has a number of equivalent carbons equaling 40, known by LLLn. Thus, this triacylglycerol is an indicator of the presence of seed oil in extra-virgin olive oil. Indeed, LLLn increases with the increase in the adulteration percentage of vegetable oils (from 1 to 10%). It ranges from 0.27 to 0.76% for the extra-virgin olive oil adulterated with soybean oil, from 0.11 to 0.69% for the extra-virgin olive oil adulterated with corn oil and from 0.05 to 0.11% for the extra-virgin olive oil adulterated with sunflower oil. The adulteration with soybean oil produces a large increase of the areas of peak LLLn because the soybean oil is the only one rich in linolenic acid.

The addition of small quantities of seed oil can be identified in the extra-virgin olive oil by the determination of the rate of LLL since the three seed oils are rich in linoleic acid (C18:2).

Similarly, the presence of soybean, sunflower and corn oils were proven by an increase of the percentage of LLL and ECN42. The extra-virgin olive oils adulterated by the sunflower, corn and soybean oils (from 1 to 10%). Having a content of LLL, they varied from 0.76 to 2.46% with sunflower oil, from 0.52 to 1.86% with corn oil and from 0.54 to 1.86% with soybean oil. Besides, having a practical ECN42, they varied from 1.26 to 3.39% with sunflower oil, from 1.33 to 2.87% with corn oil and from 1.42 to 3.59% with soybean oil. It is to be noted that olive oil is largely compared to the seed oils known by their highest linoleic acid percentages. The use of the ΔECN42 was proved to be more effective in detecting even low levels of adulteration of extra-virgin olive oil with most of the examined vegetable oils. According to the data on the fraudulent mixtures presented in Figures1, the determination of the ΔECN42 can be used as a parameter for the detection of fraud of extra-virgin olive oils with each one of the studied seed oils, 1% of sunflower (0.21>0.20), 3% of soybean (0.30>0.20) and 3% of corn (0.21>0.20) (Table 2). This finding can be attributed to the fact that the ΔECN42 is a number calculated by the combination of fatty acids and triglyceride composition. So, the difference in the composition of the triglycerides and the six fatty acids, taken into account for the calculation of the theoretical ECN42, between the initial samples and their mixtures can be expected. However, these differences do not have the expected effect on the values of the theoretical ECN42 and ΔECN42. A consequence of this is the complete lack of correlation between linoleic acid or LLL and ΔECN42 of vegetable oils and their admixtures with olive oil. These results are in agreement with those reported by Christopoulou et al.,[5] who claimed the parameter ΔECN42 is very useful and effective in the detection of adulteration of olive oils with vegetable oils.

Figure 1. Fatty Acid of pure Extra-Virgin Olive Oil (EVOO) and adulterated (EVOO) with three determinations: **a**: EVOO + (1-10%) of soybean oil; **b**: EVOO + (1-10%) of corn oil; **c**: EVOO + (1-10%) of sunflower oil.





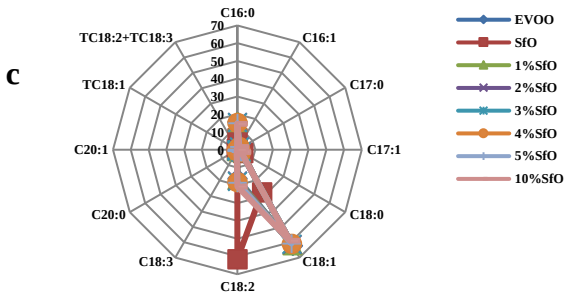


Figure 2. Triacylglycerol compositions of pure Extra-Virgin Olive Oil (EVOO) and adulterated (EVOO) with three determinations: **a**: EVOO + (1-10%) of soybean oil; **b**: EVOO + (1-10%) of corn oil; **c**: EVOO + (1-10%) of sunflower oil.

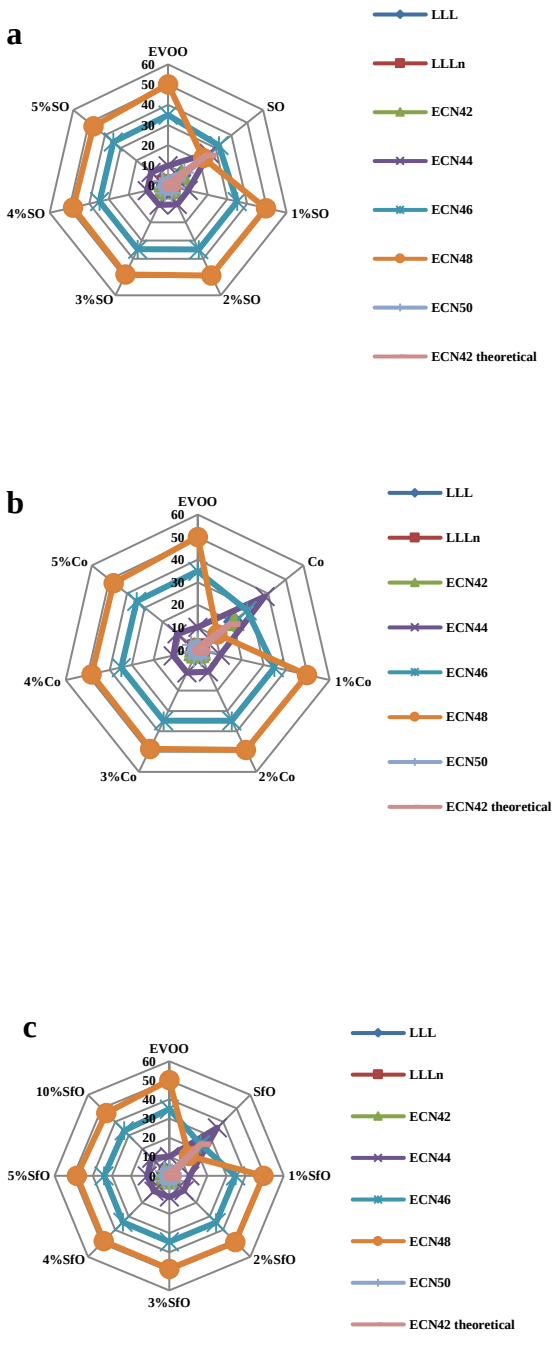


Figure 3. Trans-fatty acids of mixtures of EVOO with (**a**)soybean, (**b**)corn and (**c**)sunflower oils. Each value represents the mean of four determinations of two successive crop seasons (n=4;Standard deviation < 0.001%). EVOO: extra-virgin olive oil

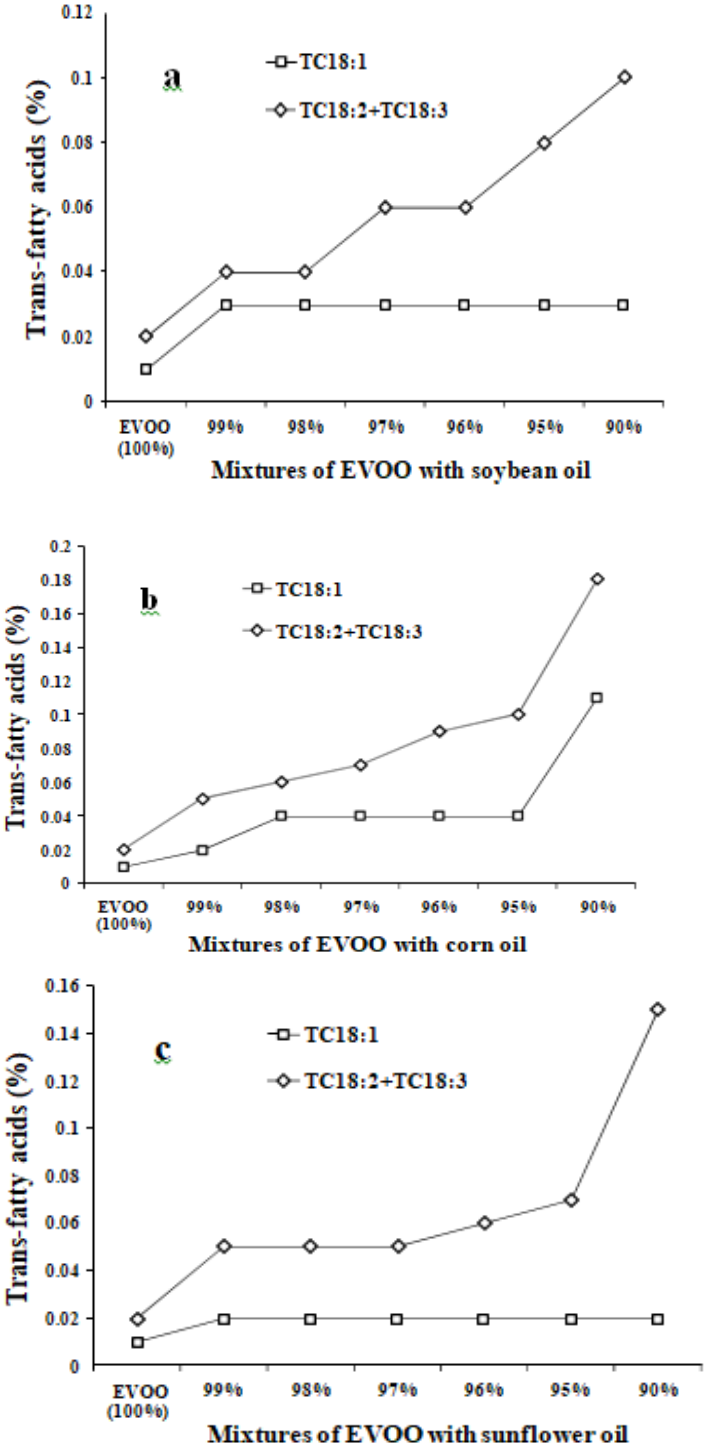


Table 1. Used Parameters for the Detection of Extra-Virgin Olive Oil Adulteration with Vegetable Oils

Type of seed oil	Used parameter for the detection of adulteration	COI threshold value	Percentage of detectable seed oil which exceeding the COI threshold value
Soybean	C18:3	1%	EVOO+5% SO (1.08%)
	ECN40	0	EVOO+1% SO (0.27)
	ΔECN42	0.20	EVOO+3% SO (0.30)
	Campesterol	4%	EVOO+10% SO (6.17%)
	Δ7-stigmastenol	0.50%	EVOO+10% SO (0.59%)
	Apparent β-sitosterol	93.00%	EVOO+10%SO (89.21%)
	∑(TC18:2+TC18:3)	0.05%	EVOO+3% SO (0.06%)
Corn	ECN40	0	EVOO+1% CO (0.11)
	ΔECN42	0.20	EVOO+3% CO (0.21)
	Campesterol	4%	EVOO+4% CO (4.06%)
	Apparent β-sitosterol	93.00%	EVOO+10%CO (91.86%)
	∑(TC18:2+TC18:3)	0.05%	EVOO+2% CO (0.06%)
Sunflower	ECN40	0	EVOO+1% SfO (0.05)
	ΔECN42	0.20	EVOO+1% SfO (0.21)
	Δ7-Stigmastenol	0.50%	EVOO+1% SfO (0.54%)
	Apparent β-sitosterol	93.00%	EVOO+5%SFO (92.89%)
	∑(TC18:2+TC18:3)	0.05%	EVOO+4% SfO (0.06%)

EVOO: extra-virgin olive oil. SO: soybean oil. CO: corn oil.
SfO: sunflower oil.

Chemometric Analysis

LDA is probably the most frequently used supervised pattern recognition method. In principle, LDA determines linear discriminant functions, which maximise the ratio of between-class variance and minimise the ratio of within- class variance. It should be noted that, whereas the principal component analysis (PCA) selects a direction that retains maximal structure among the data in a lower dimension (Figure 4), LDA selects a direction that achieves maximum separation among the given classes.[16]

Since the data structure analysis gave a good sample characterization, a classification model was built. LDA analysis was applied in order to find a predictive classification model, able to differentiate the pure extra-virgin olive oil and the adulterated olive oils.

The plot of the discriminant functions (Figure 5) obtained by LDA showed a clear discrimination between the Chemlali extra-virgin olive oil and the adulterated extra-virgin olive oils mixed with different percentages of soybean, corn and sunflower oils (1, 2, 3, 4, 5 and 10%). Discriminant function 1, which was highly related to ECN50, LLLn, C17:1 and C18:0, represent the function of minor compounds, already considered as a fingerprint for specific oil. Discriminant function 2 is highly related to C17:0, ECN42 theoretical, LLLn and C18:3. In particular, by discriminant function 1 it was possible to discriminate Chemlali extra-virgin olive oil and the adulterated extra-virgin olive oils whatever the percentage of seed oil added (Figure5). All samples were correctly discriminated using the two functions. Application of LDA, after feature selection, was sufficient to differentiate Chemlali extra virgin olive oil and all adulterated extra-virgin olive oils. The success was 100% in classification and close to 100% in prediction.

It is difficult to discriminate the adulterated EVOO with 1% of sunflower oil and the pure EVOO whereas those adulterated with other vegetables oils are clearly separated at the same level (Figure 6a). The separation improves when the percentage of adulteration increases (Figure 6b, c and d).

Compared to classical methods, this new approach of using LDA application could represent an alternative and innovative tool for faster and cheaper evaluation of extra-virgin olive oil adulteration.

Figure 4. PCA biplot of pure EVOO and adulterated EVOO based on all the analyses performed with four determinations. 1: EVOO: extra-virgin olive oil; 2: EVOO + (1-10%) of soybean oil; 3: EVOO + (1-10%) of corn oil; 4: EVOO + (1-10%) of sunflower oil.

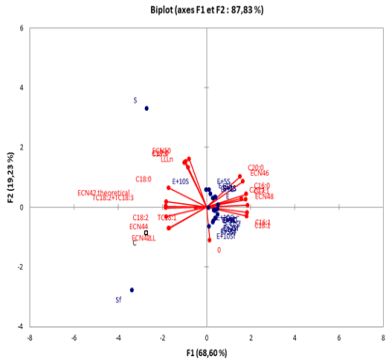


Figure 5. LDA score plot of pure EVOO and adulterated EVOO based on all the analyses performed with four determinations. 1: EVOO: extra-virgin olive oil; 2: EVOO + (1-10%) of soybean oil; 3: EVOO + (1-10%) of corn oil; 4: EVOO + (1-10%) of sunflower oil.

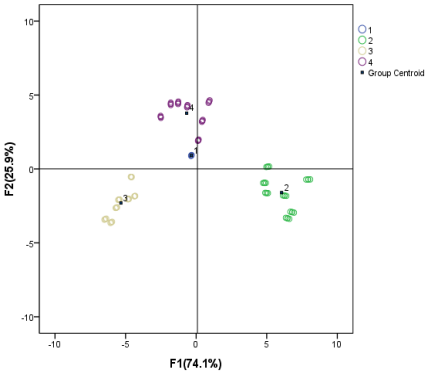
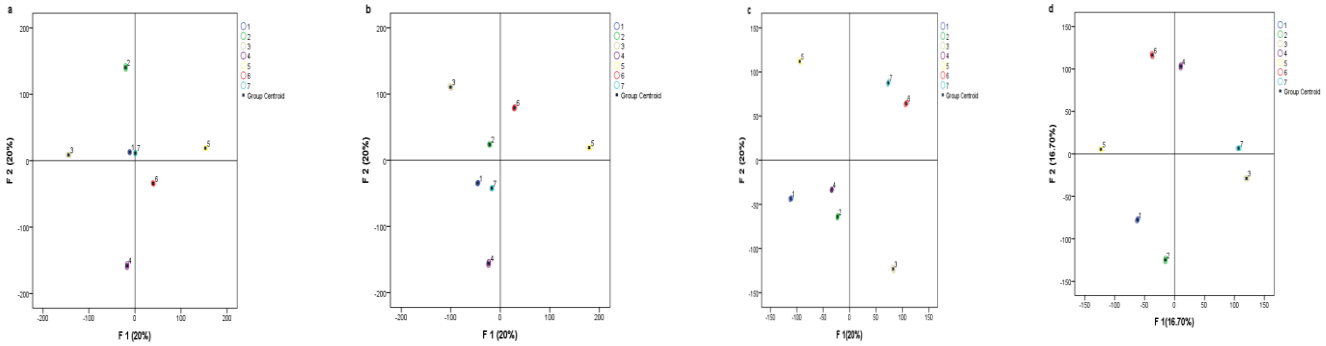


Figure 6. LDA score plot of pure EVOO, pure seed oils and adulterated EVOO at the same level based on all the analyses performed with four determinations. (**a**): EVOO + 1% of seed oil; (**b**): EVOO + 2% of seed oil; (**c**): EVOO + 5% of seed oil; (**d**): EVOO + 10% of seed oil. 1: EVOO: extra-virgin olive oil; 2: soybean oil; 3: corn oil; 4: sunflower oil; 5: EVOO + % of soybean oil; 6: EVOO + % of corn oil; 7: EVOO + % of sunflower oil.



3. Materials and Methods

Vegetable Oils

Four different extra-virgin olive oil samples were obtained from a Tunisian olive variety which is the Chemlali cultivar harvested from Sfax region (south Tunisia) during two crop seasons (2012/2013 and 2013/2014) (n=4; two different samples for each crop season).The ripening degree was the same for the four Chemlali olive samples (maturation indices were 4.5). The olive samples were collected at the beginning of December from orchards in the same neighborhood carried out with the same cultural practices. Pure soybean, corn and sunflower oils were purchased from the local market in Tunisia, used as adulterant, were checked for their authenticity by classical tests such as gas chromatography (fatty acid), with some being based on high-performance liquid chromatography (triglyceride composition). The different mixtures of extra-virgin olive oil with the

aforementioned vegetable oils (at the levels of 1, 2, 3, 4, 5 and10% w/w) were prepared.

Determination of Fatty Acids Composition

The methyl esters for the determination of the cis/ and trans-fatty acids was determined by Gas chromatograph equipped with a FID detector (HP 6890N, Agilent (Palo Alto, CA, USA)). According to the method reported by Jabeur et al., [17]

Determination of Triglycerides Composition

A 5% of the sample to be analyzed was prepared by weighing 0.25±0.001 g into a 5 mL graduated flask and dissolved in 5 mL with acetone.[18] A HP1100 chromatograpic system from Agilent (Waldbronn, BW, and Germany) equipped with a differential refractometer detector was employed. Next, the separation was carried out with a spherisorb analytical column (250 x 4.6 mm, 5 µm particle size) from Supelco (Bellefonte, PA,USA). The optimized separation conditions were conducted by isocratic elution with a 60:40 acetone/acetonitrile mixture;column temperature, 30 °C; flow rate, 1.5 mL/min and injection volume, 20 µL of the sample solution prepared as indicated above.

For the identification of TAGs, the retention times plotted in accordance with alternatively reference chromatograms obtained from soybean oil, mixture 30:70 soybean oil -olive oil and pure olive oil as described by COI.[22] It was assumed that the sum of the areas of the peaks corresponding to the various TAGs was equal to 100%, and the relative percentage of each TAGs was calculated.

It is worthwhile to note that the theoretical value of ECN42 triacylglycerols was calculated by the computer programme.

Statistical Analysis

The results were expressed as mean ± standard deviation (SD) of four measurements for the analytical determination. PCA and LDA was applied to discriminate Chemlali extra-virgin olive oil and the adulterated extra-virgin olive oils mixed with different percentages of soybean, corn and sunflower oils (1, 2, 3, 4, 5 and 10%) according to all the parameters investigated. Both, PCA and LDA plots were performed using SPSS Statistics 17.0 for Windows (SPSS Inc., 2008).

4. Conclusions

The present research, aimed at discriminate the Chemlali extra-virgin olive oils adulteration by some cheaper vegetable oils such as soybean, corn and sunflower oils, was detected using a gas chromatography in combination with liquid chromatography. According to these techniques, certain compounds found in the studied oils (triacylglycerols and fatty acids) are identified, analyzed and used for the detection of the adulteration of extra-virgin olive oils. It should also be focused on two multivariate analysis methods: principal component analysis (PCA) and linear discriminant analysis (LDA). This study has shown that LDA on chemical composition data can be used to separate the adulteration extra-virgin olive oil with the most common vegetable oils into groups.

Conflicts of Interest

The authors declare no conflict of interest.

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