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Detection of Olive Oil Adulteration with Hazelnut Oil: Analytical Challenge

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Abstract: The olive sector contributed 19% of Morocco's domestic use of edible oil. Due to its senses, compositional and nutritional characteristics, and high economic value, olive oil can lead to adulteration. This is mainly related to the difference in price between olive oil and other vegetable oils, which can create opportunities for a fraudulent substitution or admixing it with other oils. For this purpose, our study aims to detect the adulteration of 3 types of olive oil from Morocco, Tunisia, and Spain, with hazelnut oil at 5, 10, and 15%, to highlight the adulteration of the olive oil hazelnut oil. The physicochemical criteria studied to search for such adulteration are the fatty acid composition, the sterol composition, the tocopherol composition, and the triglyceride composition. This study shows that the search for olive oil adulteration should involve identifying and characterizing several purity criteria. The results obtained show that the adulteration of olive oil is detectable at 5%.

Keywords: olive oil; hazelnut oil; adulteration detection; analytical methods.

1. Introduction

Olive oil production has great socio-economic importance in Mediterranean countries. The consumer perceives olive oil as a mythical oil surrounded by symbols that give it a special place in the field of fats. However, its virgin state is considered a fruit juice and appreciated for its organoleptic and physicochemical characteristics and nutritional properties [1,2]. Olive oil is mostly composed of triglycerides (TAG), such as oleic acid (C18:1), which are responsible for its physicochemical properties [3], and health benefits compounds such as phenolic compounds and triterpenic acids [4]. Olive oil composition and properties vary depending on factors such as the olive variety, environmental conditions, and the technological methods used in processing [5].

Most of the olive oil produced in Morocco is intended for the domestic market [6]. Nevertheless, several obstacles encumber the development of this sector, namely the supply of olive oil through informal channels and traditional mills and the predominance of the wholesale market. However, producing good quality olive oil aims not only to preserve the brand image of production but also to protect the interest of consumers against health risks and adulteration [7,8]. Nevertheless, investigations have revealed the existence of sets of olive oil adulterated with oleic oils. This fraudulent practice may also cause severe health and safety problems, such as the Spanish toxic oil syndrome in 1981 [9].

Although public organizations, academic institutions, and the food industry are paying attention to preventing food fraud to have enough reliable and freely available information. Indeed, some industries do not respect the good manufacturing practices recommended by international standards and resort to the adulteration of olive oil with other oleaginous oils such as soybean oil, peanut oil, and sunflower oil [10-15].

The search for adulteration of virgin olive oils by other edible oils is a real analytical challenge. Nevertheless, their sensitivity level is not enough to detect low amounts of other oleaginous oils [16,17]. Previous work on the adulteration of virgin oils with other seed oils has led to the development of a 5% fraud detection method based on the content of campesterol [18]. In the field of fats, determinations of fatty acid and sterol composition by GPC have been used to control the purity of fats. Other complementary techniques are used to determine tocopherols and triglycerides by HPLC and stigmastadienes by GPC [19]. However, the European Commission Regulation 2568/91 [20] and International Olive Council (IOC) standards [21] address this issue through the analysis of fatty acids (FAs), triacylglycerols (TAGs), and sterols. But fraudsters can mask adulteration by removing certain compounds as in the case of destabilized seed oils. However, hazelnut oil (HO), obtained from Corylus avellana L. seeds, is often used in olive oil adulteration because of the similar chemical composition [22-24]. Despite the fact that the olive oil sector is highly regulated, it is acknowledged that there are still problems. New analytical techniques need to be developed [25, 26]. Several analytical techniques have been applied in the analysis of oil adulterated with various proportions of edible oils [16],[27-29]. A recent review highlighted the developments of analytical techniques in authentication and detecting adulteration of olive oils [7] and proposed possible solutions to safeguard the consumer and protect the olive oil market [30,31]. Nevertheless, hazelnut oil has a similar composition to olive oil in triacylglycerol, sterol, and fatty acid, and it is often used for adulteration. In this regard, our work aims to identify markers to detect adulteration of 3 types of olive oil (Moroccan olive oil (MOO), Tunisian olive oil (TOO), and Spanish olive oil (SOO)) admixed with hazelnut oil (HO) at 5, 10 and 15% (v/v). This work can provide a useful perspective for future industrial applications for olive oil quality.

2. Materials and Methods

2.1. Chemicals and reagents.

All reagents and solvents used in the current study were of analytical grade.

2.2. Samples.

The investigated oils are commercial samples purchased from retail stores in Morocco. In addition, tree vegetable olive oils were collected from several oil-producing countries (Morocco, Spain, and Tunisia) admixed with hazelnut oil at 5, 10, and 15% (v/v) rates.

2.3. Analytical methods.

Each method was carried out in triplicate for each sample. The mean values were given in the tables without the standard deviation because this value would represent only the deviation of the method and not the variation of the appropriate sample.

2.3.1. Physicochemical characterization:

The Physico-chemical parameters (free acidity, free acidity, peroxide value (PI), absorbance in UV "K_{232} and K_{270}", water content and volatile matter, and the content of impurities) are determined in accordance with the methods recommended by the C.O.I. [21].

2.3.2. Determination of the fatty acid composition.

Approximately 1 g of oils was dissolved in 1.5 ml potassium hydroxide in an ethanolic solution (0.25 N) and 10 mL of heptane. The mixture was heated under reflux for 10 minutes, followed by adding 2% of aqueous sodium carbonate solutions, and then refluxed for 3 more minutes. After the esterification, the supernatant layer was collected and completed with heptane for gas chromatographic (GC- (GC-SM, TRACE 1300-ISQ LT) analysis according to the conditions described in ISO 5508: 1990, using a VARIAN chromatograph with flame ionization detector (FID), equipped with a capillary column (CPWAX) 30 m long and 0.25 mm inside diameter. The oven temperature was set at 200 °C and that of the injector at 220 °C. The carrier gas used was helium at 1.2 mL/min, and the volume of the injection was 1µL, leakage (split on) at a ratio of 15%.

2.3.3. Determination of the sterol composition

Determination and identification of sterols present in olive oils and fraudulent blends: Briefly, 2.5 mL of olive oils were added to the potassium hydroxide solution (1M) and then heated under reflux until a clear solution was obtained. After 1 h of boiling, 100 mL of distilled water was added, and the solution was extracted three times with 25 mL of petroleum ether and washed with 15 mL of water-ethanol 90/10 (v/v). The solvent of the collected fraction was eliminated with a rotavapor. The unsaponifiable fraction was dissolved in 0.5 mL of chloroform and then deposited on 2 cm of a silica plate. The plate was pulverized by a solution of 2,7-dichlorofluorescein in the band corresponding to sterols was scraped. The sterols recovered from the plate were dissolved in 10 mL chloroform and evaporated. Then, 100 mL of pyridine hexamethyldisilazane trimethylchlorosilane (9/3/1, v/v/v) was added to the sterols and evaporated. After saponification of the sample, the residue is solubilized in heptane and injected into gas chromatography.

2.3.4. Determination of the triglyceride composition.

0.25 g of olive oil was dissolved in 5 mL of a 4.5 mL hexane / 0.5 mL diethyl ether mixture. This solution was dispensed into a cartridge of silica (C18). The fraction was collected in a 100 mL flask, flowed by solvent evaporation, and diluted with 2.5 mL acetone. HPLC analyzed the triglycerides on a column in the reverse phase of C18 (250 mm × 4.6 mm, Φ silica 5 µm), according to the IUPAC method N° 2.0 324. The HPLC device is equipped with an HP refractometric detector 1047A. The elution is carried out with a mixture (acetonitrile /acetone) (v/v) with a flow rate of 0.5 mL/min during the analysis time (90 min).

2.3.5. Determination of the tocopherol composition

To determine tocopherols, a solution of 2 g of olive oil was solubilized in 25 mL of hexane and was directly used for the high-pressure liquid chromatography HPLC (according to C.O.I.) [21].

3. Results and Discussion

3.1. Physical and chemical parameters.

The results of the physical-chemical analysis of the olive oil are presented in Table 1. They are in accordance with the requirements of the COI commercial standard [21]. Low peroxide index and specific spectrophotometric values ($K_{232} \leq 2.5$, $K_{270} \leq 0.22$, $\Delta K \leq 0.01$), show low values, which are indicators not only of quality but also authenticity [32].

Table 1. Physicochemical analysis of olive oil (Ia=Acidity index in % free acidity, Ip= Peroxide value (meq O_2/Kg); EMV= Water and volatile matter (%)).

	Olive Oil	Norme C.O.I
Ia (%)	0,3	≤ 0,8
Ip	1,473	≤ 20
K_{270}	0,19	≤ 0,22
K_{232}	1,97	≤2,50
ΔK	0.00	≤ 0,01
Impurities	0.021	≤ 0,10
EMV (%)	0.08	≤ 0,20

3.2. Physico-chemical constants.

The main physicochemical constants of olive and hazelnut oils were measured by standard methods [33]: density at 20°C, viscosity at 20°C, refractive index, saponification index, and unsaponifiable matter. Indeed, the solidification point of olive oil is variable compared to hazelnut oil. These results show that the mixture of two oils of similar composition can be difficult to detect, which led us to use analytical methods to detect such adulteration.

3.3. Fatty acid composition.

The fatty acid composition of the different olive oil samples was determined after methylation of the oil and analysis of the methyl esters by gas chromatography as well as the fatty acid composition according to the standards of the International Olive Council (I.O.C) [21] are presented in Table 2. The official bodies have established limits concerning the content of fatty acids in olive oil. These limits are used to discriminate between olive oil and other vegetable oils. Moreover, olive oil and hazelnut oil are "oleic and linoleic" types and are characterized by fatty acids such as oleic acid (C18:1), linoleic acid (C18:2), palmitic acid (C16:1) and stearic acid (C18:0)).

Table 2. Fatty acid composition (area %), of (Moroccan MOO, Tunisian TOO, and Spanish SOO) olive oil samples, hazelnut oil HO and the International Olive Oil Council (COI).

Fatty acids %	M.O.O.	T.O.O.	S.O.O.	H.O.	C.O.I.
C14 :0	-	-	-	-	-
C16 :0	9.1± 0.015	17.5± 0.015	10.7± 0.015	5.46± 0.017	7.5-20
C16 :1	0.7± 0.02	2.2± 0.05	0.8± 0.02	0.12± 0.05	0.3-3.5
C18 :0	3.1± 0.005	2.6 ± 0.005	3.45± 0.005	2.57± 0.001	0.5-5
C18 :1	73.1± 0.001	58.4±0.001	77.6± 0.001	83.6± 0.001	55-83
C18:2	11.8± 0.003	17.5± 0.002	5.39± 0.002	7.50± 0.003	3.5-21
C18 :3	0.94± 0.001	0.63± 0.001	0.6± 0.001	0.06± 0.001	≤0.9
C20 :0	0.3± 0.015	0.4± 0.015	0.4± 0.013	-	≤0.6

Each data in the table are expressed by mean values ± SD of fatty acids (n = 2) and physical and chemical indices (n = 3).

Table 3. Fatty acid composition of Moroccan olive oil (MOO), Tunisian olive oil (TOO), and Spanish olive oil (SOO)) admixed with hazelnut oil (HO) at rates of 5, 10 and 15% (v/v).

Fatty acid	MO.O	Mixture (M.O.O./H.O.)			T.O.O	Mixture (T.O.O./H.O.)			S.O.O	Mixture (S.O.O./H.O.)		
		5%	10 %	5%		5%	10 %	15%		5%	10 %	15%
C16 :0	9.1± 0.015	9.1± 0.017	8.0± 0.010	16.3± 0.01	17.5± 0.015	16.3± 0.015	15.7± 0.015	16.9± 0.015	10.7± 0.015	10.63± 0.015	10.35± 0.015	10.11± 0.015
C16 :1	0.7± 0.02	0.8± 0.02	0.7± 0.02	0.2± 0.02	2.2± 0.05	2.1± 0.05	1.9± 0.05	2.2± 0.05	0.8± 0.02	0.78± 0.05	0.76± 0.05	0.73± 0.05
C18 :0	3.1± 0.005	2.9± 0.005	2.8± 0.005	2.5± 0.005	2.6 ± 0.005	2.5± 0.005	2.5± 0.005	2.5± 0.005	3.45± 0.005	3.3± 0.005	3.31± 0.005	3.34± 0.005
C18 :1	73.1± 0.001	73.1± 0.001	73± 0.001	60.7± 0.001	58.4±0. 0001	60.7± 0.001	61.9± 0.001	59.4± 0.001	77.6± 0.001	77.8± 0.001	78.17± 0.001	78.6± 0.001
C18:2	11.8± 0.003	11.9± 0.002	11.7± 0.002	16.7± 0.003	17.5± 0.002	16.7± 0.001	16.2± 0.002	17.3± 0.002	5.39± 0.002	5.56± 0.001	5.65± 0.001	5.7± 0.001
C18 :3	0.94± 0.001	0.9± 0.001	0.9± 0.001	0.6± 0.001	0.63± 0.001	0.6± 0.001	0.5± 0.001	0.6± 0.001	0.6± 0.001	0.6± 0.0015	0.6± 0.0015	0.6± 0.0016
C20 :0	0.3± 0.015	-	-	0.4± 0.015	0.4± 0.015	0.4± 0.015	0.4± 0.015	0.4± 0.015	0.4± 0.013	0.4± 0.013	0.4± 0.0128	0.4± 0.013

Olive oil shows a variation in its fatty acid composition compared to hazelnut oil. These variations are well established in the case of Tunisian olive oil, which generally has a rate of linoleic acid (C18:2) higher than the average, which consequently reduces the rate of oleic acid (C18:1). The detection of olive oil adulteration is done by the measurements of their fatty acid composition according to the Codex Alimentarius standards [34]. Table 3 shows the fatty acid composition of olive oil admixed with hazelnut oil at 5, 10, and 15% (v/v). In addition, all acid ratios between oil samples were not significantly different ($P < 0.05$). The measurements indicated a great similarity in the fatty acid composition of the three oils. However, the composition of fatty acids through the percentage of linoleic acid does not highlight a falsification of olive oil with hazelnut oil. Based on the above observations, determining fatty acids may not be used to detect the adulteration of olive oil with hazelnut oil.

3.4. Sterol compositions.

The quantitative analysis of sterols is one of the most promising methodologies that have been implemented and validated by Mariana in 2006 [35] and has become a powerful tool to assess edible oil purity. Likewise, the steroidal fraction of olive oil was a distinct fingerprint and a very useful parameter for detecting adulterations or verifying authenticity [36]. According to the literature, this analysis was used to highlight the additions of less than 15% of hazelnut oil in virgin olive oil [19,37]. Further, the sterol composition of olive oil was (98-184 mg/100g) which are intermediate value compared to the sterol composition of hazelnut oil (75-95 mg/100g) [38]. In this work, the sterol composition of olive oil was obtained by fractionating the unsaponifiable matter and determined by gas chromatography is presented in Table 4. The obtained results show high contents of β-sitosterol up over 90% of total sterol content, while no significant differences between the percentage of sterols composition and the value is far above 1000 mg/kg demanded by the standards of the International Olive Council (I.O.C.).

Table 4. Sterol compositions of (Moroccan, Tunisian and Spanish) olive oil, hazelnut oil, and the International Olive Oil Council (COI).

Sterols	M.O.O.	T.O.O.	S.O.O.	H.O.	C.O.I.
Cholesterol	0.5± 0.0020	0.2± 0.0025	0.8± 0.0027	0.3± 0.005	≤0.5
Campesterol	3.5± 0.0335	3.2± 0.0330	3.2± 0.00315	5.3± 0.005	≤4

Sterols	M.O.O.	T.O.O.	S.O.O.	H.O.	C.O.I.
Stigmasterol	1.2± 0.0290	0.6± 0.0255	0.6± 0.0260	0.9± 0.0170	≤4
Chlerosterol	0.88± 0.0501	0.88± 0.0519	0.88± 0.0521	0.34± 0.0520	≤4
ß-sitosterol	85.5± 0.2230	79.9± 0.3100	86.1± 0.240	82.8± 0.110	3.5-21
Δ-5- avenasterol	7.2± 0.250	11.2± 0.250	6.8± 0.305	6.3± 0.140	+
Δ-7-stigmasterol	0.4± 0.001	0.35± 0.001	0.4± 0.001	1.6± 0.001	+
Δ-7-avenasterol	0.3± 0.006	0.6± 0.006	-	0.6± 0.005	≤93.0

The measurements in Table 5 demonstrate the sterol composition of admixtures (Moroccan, Tunisian and Spanish) of olive oil with hazelnut oil. According to the obtained results, there is a gradual increment of the values of campesterol and **Δ** 7- stigmasterol from olive oils to hazelnut oils. In contrast, the values of **Δ** 7-avenasterol do not show significant differences. The sterol compositions are in accordance with the literature and with the standards of the International Olive Council (IOC) [21,37]. However, the proportion of ß-sitosterol is almost similar in (M.O.O) at 5, 10, and 15% (v/v), accompanied by small amounts of campesterol and Δ-5-avenasterol in (T.O.O). Nevertheless, some differences appeared with significant levels of Δ-7-avenasterol in Spanish olive oil admixed with hazelnut oil. As described, these significant levels of Δ-7-avenasterol can highlight the adulteration of Spanish olive oil at levels of at least 5%. Moreover, this method will not detect smaller quantities of falsification for all varieties of olive oil.

Table 5: Sterols compositions of Moroccan olive oil (MOO), Tunisian olive oil (TOO), and Spanish olive oil (SOO)) admixed with hazelnut oil (HO) at rates of 5, 10, and 15% (v/v).

Sterol	M.O.O.	Mixture (M.O.O./H.O.)			T.O.O.	Mixture (T.O.O/H.O.)			S.O.O.	Mixture (S.O.O./H.O.)		
		5%	10 %	15%		5%	10 %	15%		5%	10 %	15%
Cholesterol	0.5± 0.0020	0.2± 0.0021	0.25± 0.0022	0.5± 0.0020	0.2± 0.0025	0.20± 0.0025	0.46± 0.0026	0.6± 0.0024	0.8± 0.0027	0.32± 0.0219	0.35± 0.0010	0.41± 0.0021
Campesterol	3.5± 0.0335	3.5± 0.052	3.65± 0.0336	3.7± 0.0333	3.2± 0.0330	3.25± 0.0292	3.36± 0.029	3.4± 0.027	3.2± 0.00315	3.42± 0.0335	3.42± 0.0325	3.49± 0.0305
Stigmasterol	1.2± 0.0290	1.16± 0.0291	1.16± 0.0292	1.14± 0.0292	0.6± 0.0255	0.6± 0.0290	0.6± 0.0291	0.6± 0.0292	0.6± 0.0260	0.83± 0.0292	0.87± 0.0290	0.87± 0.0293
Chlerosterol	0.88± 0.0501	0.15± 0.0502	0.15± 0.0429	0.16± 0.0501	0.88± 0.0519	0.16± 0.0501	0.16± 0.0502	0.17± 0.0501	0.88± 0.0521	0.15± 0.0501	0.16± 0.0501	0.16± 0.0501
ß-sitosterol	85.5± 0.2230	85.2± 0.2501	84.9± 0.261	83.8± 0.2232	79.9± 0.3100	81.1± 0.3100	81.1± 0.3100	79.9± 0.3100	86.1± 0.240	86.6± 0.239	85.2± 0.241	85.5± 0.238
Δ-5-avenasterol	7.2± 0.250	7.3± 0.249	7.4± 0.247	7.45± 0.249	11.2± 0.250	11.0± 0.251	11.4± 0.251	11.8± 0.250	6.8± 0.305	6.1± 0.300	6.5± 0.291	6.5± 0.292
Δ-7-stigmasterol	0.4± 0.001	0.4± 0.001	0.41± 0.001	0.43± 0.001	0.35± 0.001	0.41± 0.001	0.45±± 0.001	0.45± 0.001	0.4± 0.001	0.5± 0.001	0.59± 0.001	0.6± 0.001
Δ-7-avenasterol	0.3± 0.006	0.3± 0.006	0.3± 0.006	0.3± 0.006	0.6± 0.006	0.50± 0.006	0.51± 0.006	0.55± 0.006	-	0.5± 0.006	0.4± 0.006	0.4± 0.006

3.6. Tocopherol compositions.

Tocopherols are important functional components in foods. They have vitamin and antioxidant activity. Several methods are reported in the literature to determine tocopherol compositions in vegetable oil [38-41]. As presented in Table 6, α -tocopherol is the main tocopherol of all olive oil, and Hazelnut oil possessed the highest α-tocopherol content (456.10mg/kg), followed by significant levels of the (β+γ)-tocopherols concentrations.

Table 6. Tocopherol compositions of (Moroccan, Tunisian and Spanish) olive oil samples, and hazelnut oil.

	M.O.O	T.O.O	S.O.O	H.O
α- tocopherol	46.7	188.2	181	**456.10**
β- tocopherol	1.37	4.12	2.73	19.23
γ- tocopherol	4.12	2.74	21.98	74.18
δ- tocopherol	-	-	-	4.12

δ-tocopherol has not been detected in any olive oil samples and was detected in high concentrations in Hazelnut oil (4.12mg/kg). However, tocopherol compositions were used for oil adulteration detection. TingShi et al. [42] provided an accurate quality assessment for camellia oil adulterated with common vegetable oils based on the tocopherol compositions.

The tocopherol compositions of admixtures (Moroccan, Tunisian and Spanish) olive oil with hazelnut oil at rates of 5, 10, and 15% (v/v) are shown in Table 7. The obtained results indicate that the concentration of α-tocopherol, β- tocopherol, and γ- tocopherol increases considerably with a significant concentration of δ-tocopherol in adulterated olive oil. However, determining the tocopherol content allows highlighting the adulteration of the olive oil by the hazelnut oil.

Table 7. Tocopherols composition of Moroccan olive oil (MOO), Tunisian olive oil (TOO), and Spanish olive oil (SOO)) admixed with hazelnut oil (HO) at rates of 5, 10 and 15% (v/v).

Tocopherols	M.O.O	Mixture (M.O.O/HO)			T.O.O	Mixture (T.O.O/HO)			S.O.O	Mixture (S.O.O/HO)		
		5%	10 %	15%		5%	10 %	15%		5%	10 %	15%
α-tocophérol	46.7	56.9± 0.002	66.1± 0.002	76.4± 0.002	188.2	190.2± 0.0025	200.2± 0.0026	220.5± 0.0024	181	189.2± 0.0219	205± 0.0010	221.6± 0.0021
β- tocophérol	1.37	3.5± 0.050	3.65± 0.011	3.7± 0.004	4.12	5.03± 0.0292	5.80± 0.029	5.91± 0.007	2.73	3.51± 0.0335	3.98± 0.0305	4.09± 0.0305
γ- tocophérol	4.12	4. 52± 0.0291	4.93 0.0292	5.14± 0.0292	2.74	0.6± 0.0290	0.6± 0.0291	0.6± 0.0292	21.98	21.83± 0.0292	21.91± 0.0290	22.07± 0.0293
δ- tocophérol	-	3.21± 0.001	3.42± 0.001	3.51± 0.001	-	3.65± 0.001	3.90± 0.002	4.01± 0.001	-	3.95± 0.001	4.16± 0.001	4.35± 0.001

3.7. Triglyceride composition.

The triglycerides of the different olive oil samples are done according to their molecular weight, degree of establishment, and a number of partitions on high-pressure liquid chromatography HPLC in reversed phase. The analysis allowed the separation of the individual triglycerides presented in Table 8, which indicates the predominance of OOO, POO, OLO, LOL, and PLO. These results are in accordance with data from the literature [18].

Table 8. Triglyceride composition of (Moroccan, Tunisian and Spanish) olive oil and hazelnut oil.

Sample /TG	HO	MOO	TOO	SOO
LLL	1.16	0.11	0.14	0.14
OLL	0.10	0.51	0.52	0.57
LOL	3.53	4.27	4.41	4.53
OLO	0.1	1.38	1.69	1.63
PLO	5.53	7.15	6.80	6.95
OOO	81.67	44.26	41.15	45.21
POO	19.01	19.90	20.39	20.56
POP	1.36	1.67	1.52	2.01

The detection of admixed olive oil with hazelnut oil using the absolute values of triglyceride can not be used. A further step was looking to use the absolute difference between the experimental values of triacylglycerols (TAGs) with equivalent carbon number 42 (ECN42HPLC) and the theoretical value of TAGs with an equivalent carbon number 42 (ECN42theoretical). Experimental and theoretical differences proved to be more effective in detecting low levels of adulteration of olive oil with vegetable oils [38,43].

Therefore, For these reasons, ECN42 and ΔECN42 are shown in Table 9, and the difference between the theoretical and experimental ECN42 values (ΔECN42) in adulterated oils was higher than that in olive oils.

Certain researchers have pointed out the importance of the parameter (LLL/ECN42)*100, in foretelling the geographical origin of the olive oil, as well as for the

detection of adulteration of olive oil with certain vegetable oils [39]. This parameter is based on the low LLL content of the olive oil in comparison to that of the admixed olive oil. The value of this parameter in olive oil was found to range from 15.0 to 60 (mean value of 35). However, the established limit of ΔECN42 and the use of the parameter (LLL/ECN42)*100, are not suitable for detecting percentages lower than or equal to 5% of hazelnut oil in olive oil. According to official standards, the ΔECN42 of olive oil was found to be different statistically ($P < 0.05$) from adulterated oils [43]. The obtained values showed the adulteration of Moroccan oil and Tunisian oil at 10% and Spanish oil at 5%, which indicates the predominance of triglycerides in olive oil. Our results are in accordance with the literature [43-45]. According to the result presented in Table 9, the determination of the ΔECN42 can be used as a parameter for detecting adulteration of Moroccan oil and Tunisian oil at 1, the level of 10%, and Spanish oil at the level of 5%.

Table 9. Triglyceride composition of Moroccan olive oil (MOO), Tunisian olive oil (TOO), and Spanish olive oil (SOO)) admixed with hazelnut oil (HO) at rates of 5, 10 and 15% (v/v):

Triglyceride	H.O.	M.O.O.	Mixture			T.O.O	Mixture			S.O.O	Mixture		
			5%	10 %	15%	.	5%	10 %	15%	.	5%	10 %	15%
LLL	0.51± 0.0020	0.11± 0.0020	0.11± 0.0020	0.11± 0.0020	0.11± 0.0020	0.14± 0.0020	0.14± 0.0020	0.14± 0.0020	0.14± 0.0020	0.12± 0.0020	0.12± 0.0020	0.12± 0.0020	0.12± 0.0020
ECN42THO	0.84± 0.001	0.80± 0.001	0.80± 0.001	0.79± 0.001	0.76± 0.001	0.80± 0.001	0.72± 0.001	0.79± 0.001	0.66± 0.001	0.22± 0.001	0.22± 0.001	0.24± 0.001	0.34± 0.001
ECN42HPLC	0.83± 0.0290	0.87± 0.0290	0.72± 0.0290	0.76± 0.0290	0.49± 0.0290	0.87± 0.0290	0.80± 0.0290	0.49± 0.0290	0.76± 0.0290	0.23± 0.0290	0.24± 0.0290	0.43± 0.0290	0.44± 0.0290
ΔECN42	-0.01± 0.001	0.07± 0.001	-0.08± 0.001	-0.2± 0.001	-0.3± 0.001	0.07± 0.001	0.08± 0.001	-0.3± 0.001	0.1± 0.001	0.01± 0.001	0.02± 0.001	0.19± 0.001	0.1± 0.001
(LLL/ECN42)*100	61.4± 0.001	12.64± 0.001	15.27± 0.001	14.74± 0.001	22.44± 0.001	16.09± 0.001	17.5± 0.001	28.57± 0.001	18.42± 0.001	52.17± 0.001	50± 0.001	27.90± 0.001	27.27± 0.001
L1 ECN42	0.070± 0.006	0.07± 0.006	0.08± 0.006	0.30± 0.006	0.30± 0.006	0.07± 0.006	0.08± 0.006	0.30± 0.006	0.30± 0.006	0.01± 0.006	0.02± 0.006	0.19± 0.006	0.21± 0.006
L2 ECN42	09± 0.006	0.087± 0.006	10± 0.006	37.97± 0.006	39.47± 0.006	0.087± 0.006	10± 0.006	37.97± 0.006	39.47± 0.006	4.34± 0.006	8.33± 0.006	87.5± 0.006	87.5± 0.006
ECN44 THO	5.3± 0.001	5.98± 0.001	6.03± 0.001	5.95± 0.001	5.82± 0.001	5.98± 0.001	6.03± 0.001	5.95± 0.001	5.82± 0.001	2.52± 0.001	2.57± 0.001	2.59± 0.001	2.56± 0.001
ECN44 HPLC	6.3± 0.001	6.31± 0.001	6.20± 0.001	4.20± 0.001	4.65± 0.001	6.31± 0.001	6.2± 0.001	4.20± 0.001	4.65± 0.001	9.10± 0.001	3.03± 0.001	3.32± 0.001	3.98± 0.001
L1 ECN44	0.03± 0.001	0.17± 0.001	0.17± 0.001	1.75± 0.001	1.17± 0.001	0.33± 0.001	0.17± 0.001	1.75± 0.001	1.17± 0.001	0.51± 0.001	0.46± 0.001	0.73± 0.001	1.42± 0.001
L2 ECN44	0.5± 0.001	0.05± 0.001	0.03± 0.001	29.41± 0.001	20.10± 0.001	0.05± 0.001	0.03± 0.001	29.41± 0.001	20.10± 0.001	20.23± 0.001	1.710± 0.001	20.10± 0.001	0.016± 0.001

Parameters calculated by the triglycerides: ΔECN42 = ECN42HPLC – ECN42Théor; (LLL/ECN42)*100

4. Conclusions

The control of quality and adulteration of olive oil has developed as it is important to consumers. Different analytical methods and parameters are used to detect adulteration of olive oil. In the present study, we focus on detecting olive oil adulteration with hazelnut oil by using fatty acid, sterol compositions, and ΔECN42 values. According to our results, it can be said that fatty acids and sterol compositions are not very effective in detecting adulteration of olive oil. Furthermore, the determination of the ΔECN42 can be used to detect adulteration of Moroccan and Tunisian oil at the level of 10% and Spanish oil at the level of 5%. On the other hand, δ-tocopherol seems to be the target analyte for the identification of adulteration in admixed olive oil. Consequently, this approach can be useful as a source of information for detecting the adulteration of olive oil with other edible oils.

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Conflicts of Interest

The authors declare that they have no conflicts of interest in relation to this article.

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