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# Comprehensive study on the characteristics and authenticity of Egyptian monovarietal coratina virgin olive oil

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**Abstract:** Coratina virgin olive oil obtained from one variety (monovarietal) cultivated in Giza, Egypt is usually unique, valuable and has high price, which make it vulnerable to adulteration by cheaper oils. The complete profiles of tocopherols, phytosterols and fatty acid composition of the Egyptian monovarietal coratina extra virgin olive oil (CEVOO) were determined using HPLC and GLC to reveal the exact features of this oil which is unique among the other oils. Also, it is planned to determine the authenticity of CEVOO adulterated with soybean and sunflower oils. Models of soybean or sunflower oils as adulterants in CEVOO at levels of 5, 10, and 20% were prepared. The CEVOO is characterized by higher content of α-tocopherol (97.77%). The results showed that gamma-tocopherol content could be used as a parameter for detection of CEVOO adulterated with soybean oil at levels as low as 5%. In addition, the marked decreases in alpha- / gamma-tocopherol as well as PUFA/alpha-tocopherol ratios indicate the adulteration. It was noticeable that CEVOO is characterized by the high amount of squalene (3471.8 µg/g). Moreover, the phytosterol profile is almost decisive in clarifying the adulteration of CEVOO with cheaper oils thus, 5% sunflower oil could be detected by presences of 7-stigma- and 7-avenasterol. Meanwhile, the increase in campesterol indicated the presence of other oils. In addition, authenticity factor and the ratio of β-sitosterol/campesterol+ stigmasterol can indicate the presence of adulteration of CEVOO at levels as low as 5%.The highest monounsaturated fatty acid content in CEVOO was 73.3%, which is mainly due to the predominant presence of oleic acid. Linoleic / linolenic acids ratio could be used as proof for adulteration of CEVOO. These results through light on the characteristics of Egyptian monovarietal CEVOO as well as the detection of its adulteration.
**Key words**: Monovarietal coratina extra virgin olive oil, tocopherols, phytosterols, fatty acids, adulteration, authenticity.

## Introduction

Virgin olive oil (VOO) is recognized as worldwide and it is an important ingredient in the Mediterranean diet for its nutritional value and pleasant flavor have contributed to an increase in consumption of it. The nutritional value of VOO comes from high levels of oleic acid and phenolic compounds antioxidant (Jiang *et al.,* [1,] Abdel-Razek *et al.,* [2,] De Nino *et al.,* [3]), whereas the volatile compounds is affected strongly the aroma of oil (Angerosa [4]). Olive oil quality can be recognized from commercial, nutritional or sensorial perspectives (Duran [5]).

With increasing consumer demand for high-quality virgin olive oil, oil obtained from one variety (monovarietal) cultivated in certain region, now it is available in the market. The purity and quality parameter is related with specific region and has increasing interest in the origin of geographical area. Some designation for olive oil, namely, Protected Denomination of Origin (PDO) and Protected Geographical Indication (PGI) were established to protect high-quality agricultural products. These labeling mean that the product is produced, processed, or prepared in a certain geographical area with typical characteristics linked to natural factors and environment of these regions (Babcock and Clemens[6,] Ozen *et al*., [7]). PDO or PGI olive oils are expensive in the market; therefore, can be subjected to adulteration. Adulteration of high-priced monovarietal olive oils is of concern to the food industry due to economical and health benefits. The effect of different levels of bio-fertilizer on fruit set, yield, fruit quality and oil properties of monovarietal Manzanillo olive trees grown in Ismailia governorate Egypt were studied (Abd-Alhamid *et al*., [8]). Also, different olive oil varieties were studied from two different areas of Egypt, Siwa Oasis and Giza city, who found that Coratina, Arbequina and Koroneiki coming from Giza city showed a higher perception of bitterness, spicy and fruity as well as higher content of total tocopherols than the oils of Maraqi and Wattagen coming from Siwa Oasis (Benincasa *et al.*, [9]). This could be explained by considering that Giza city, located on the west bank of the Nile River with an arid climate but also with high humidity due to the River Nile's Valley effects, demonstrated excellent nutritional characteristics in terms of antioxidant compounds. This monovarietal olive oil was targeted to cheat with other common and cheaper oils such as sunflower and soybean oils.

Consequently, there is a need to evaluate the authenticity of Egyptian monovarietal olive oil. Several methodologies have been suggested for controlling adulteration of virgin olive oils with other edible oils (Christopoulou [10,] Cercaci etal [11], Oliveros *et al.*, [12], Pena *et al.*, [13], Poulli *et al.*, [14]).

There is a lack of data about the monovarietal coratina olive oil cultivars in Egypt. This work firstly aimed to study the major and minor phytonutrient components of the Egyptian monovarietal Coratina olive oil (CEVOO). Secondly, detect the adulteration of CEVOO with cheaper vegetable oils (soybean and sunflower). It was planned to prepare the simulated adulterated CEVOO with soybean or sunflower oil, at levels of 5, 10 and 20% w/w. These models were analyzed for profiles of tocopherols, phytosterols and phytostanols as well as fatty acid composition using HPLC and GLC respectively which can help to detect precisely olive oil adulteration.

## Materials and methods

### Materials

### Monovarietal coratina olive oil preparation

Coratina olive fruits were processed in Giza geographical area by Wady Food Factory, Giza, Egypt, with two phases extraction. It is noteworthy that the Egyptian monovarietal coratina olive fruits were cultivated in Giza, Egypt. In fact, Giza, the third largest city in Egypt, is located on the west bank of the Nile River. Giza experiences an arid climate, but often with high humidity due to the River Nile's Valley effects.

### Other oils

Refined, bleached and deodorized soybean and sunflower oils were kindly supplied by Cairo for Oil and Soap Factory, Giza, Egypt.

### Chemicals

All solvents HPLC grade, 1M methanolic KOH, sterol standards and anhydrous pyridine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Standards of tocopherols were obtained from Calbiochem-Novobiochem (San Diego, CA, USA), FAME standards and Sylon BTZ was purchased from Supelco (Bellefonte, PA, USA).

**Preparation of adulterated monovarietal olive oil**

Five, 10 and 20% w/w from sunflower or soybean oils as adulterants were accurately weighed then added to CEVOO for making a mixture. The prepared mixtures were ultrasonicated for 15 minutes to ensure thorough mixing of the oils.

**The prepared samples were accurately weighed (500 mg) and analyzed as follows:**

Tocopherol analysis was carried out by HPLC according to Balz *et al.,* [15,] Hassanien *et al.,* [16]. Phytosterol content and composition were determined by GC following the procedure described by AOCS Official Method AOCS [17].

Methyl esters of fatty acids (FAME) were prepared according to AOCS Official Method AOCS [18] and analyzed by GLC (Hassanien *et al.,* [16]).

Calculated oxidizability (COX) value: The Cox value of the oils was calculated by applying the formula proposed by Fatemi and Hammond [19].

Cox value={[C18:1(%)]10.3+[C18:2(%)]21.6+[C18:3(%)]}/100.

**Statistical analysis**

Results are presented as the mean ± standard deviation from three replicates of each experiment.

## Results and Discussion

Original Egyptian monovarietal CEVOO and adulterated it with addition 5, 10, or 20% by wt from sunflower (SNF) or soybean (SBO) oils as adulterants were studied. It is known that tocopherols are minor constituents in vegetable oils and their composition may be proof for oils. Tocopherols are hetero acid compounds with high molecular weight that are namely, α, β, γ, and δ-tocopherols. Table 1 represents tocopherol content of pure oils and simulated adulteration of the CEVOO with SBO and SNF. α-Tocopherol is unique characteristic of CEVOO (97.77%) similar to SNF(96.75 % ) of the total tocopherol. These results agree with Benincasa *et al.,* [9], who found that Egyptian CEVOO have α-tocopherol more than 95%. As tocopherol composition of SBO contains mainly γ- and δ-tocopherols (40.9 and 34.67% respectively). It was noticed that the level of α-tocopherol not markedly changed in case of addition of SBO to CEVOO. While, δ-tocopherols, not found in CEVOO, it appears at the level of 5.52, 9.96 and 16.71%, in the simulated adulterated CEVOO with SBO at 5, 10 and 20% w/w respectively. Also, it was found that an increase in the amounts of γ – tocopherol from 0.94% for CEVOO to 7.63, 12.16 and 20.2% in admixture with SBO. It is evident that the increase in amounts of γ-tocopherol and appearance of δ- tocopherol in admixture confirmed that there is adulteration with SBO. On the other side, mixture of CEVOO with SNF, it was noticeable increases in α-tocopherol, but no marked changes in β- and γ-tocopherols at any ratios. It is useful to use the ratio α-/ γ– tocopherol for differentiation between pure oils and mixed oils (Rossell *et al.,* [20]). Thus, the ratio α-/ γ– tocopherol amounted to 103.5 in CEVOO and this ratio dramatically decreased to 11.35, 6.33, 3.12% in case of addition SBO. Meanwhile, it reaches to 101.95, 90.3 and 84.38 % in case of admixture with SNF at 5, 10 and 20%w/w respectively. It could be noticed that the ratio α-/ γ- tocopherol was completely different from that of CEVOO depending on the amount of each oil added to the others. In addition, another ratio PUFA/ α-tocopherol was also proposed to determine oil purity (Alfin-Slater *et al.,* [21]).The ratio in CEVOO was 0.49 and it increased to 0.92,1.13 and 1.33after the addition of SBO and to 0.64, 0.68 and 0.77 in case of added SNF at 5, 10 and 20%w/w respectively. These results were supported by Deiana *et al.,* [22] , who reported that tocopherols contribute to the antioxidant properties of olive oil and their profile and composition are criteria of purity. Finally, it can be seen from the results, that the detection of δ- tocopherols in the mixture confirmed the presence of SBO as adulterant as well as higher values of γ-tocopherols.

**Table 1: Tocopherol content of pure oils and simulated adulteration of the CEVOO with SBO and SNF**

| Oil Samples | Tocopherols content (mg/100g ) | | | | Ratios and total Tocopherols | | |
|---|---|---|---|---|---|---|---|
| | α-Toc | β-Toc | γ-Toc | δ-Toc | α-Toc/ γ-Toc | PUFA / α-Toc | Total Toc. (mg/100g) |
| **CEVOO** | **20.70**± 0.31 | **0.27**± 0.03 | **0.20**± 0.06 | - | **103.5** | **0.49** | **21.17** |
| **100% SBO** | **18.29**± 0.24 | **0.61**± 0.21 | **31. 8±** 0.14 | **26.95**± 0.16 | **0.57** | **3.24** | **77.63** |
| **5% SBO** | **20.54**±0.0 | **0.03**±0.02 | **1.81**±0.14 | **1.31**±0.08 | **11.35** | **0.92** | **23.70** |
| **10% SBO** | **20.31±**0.24 | **0.31**±0.04 | **3.22**±0.25 | **2.64**±0.06 | **6.33** | **1.13** | **26.48** |
| **20% SBO** | **20.09**±0.11 | **0.32**±0.08 | **6.54**±0.25 | **5.41**±0.04 | **3.12** | **1.33** | **32.36** |
| **100% SNF** | **74.66**± 0.13 | **1.41**± 0.15 | **1.09**± 0.08 | - | **68.5** | **0.79** | **77.16** |
| **5% SNF** | **23.45**±0.14 | **0.33**±0.06 | **0.24**±0.06 | - | **101.95** | **0.64** | **24.02** |
| **10% SNF** | **26.19**±0.16 | **0.37**±0.04 | **0.29**±0.07 | - | **90.3** | **0.68** | **26.85** |
| **20% SNF** | **30.38**±0.18 | **0.51**±0.06 | **0.36**±0.09 | - | **84.38** | **0.77** | **31.25** |

CEVOO: Coratina extra virgin olive oil, SBO: soybean oil, SNF: sunflower oil, **Toc**: tocopherol.

The compositional distributions of phytosterols as minor constituents in certain vegetable oils have been used for their identification. Hence, phytosterols in oils are often used as markers for the assessment of adulterated oils. From the data recorded in Table 2, it was noticeable that CEVOO is characterized by the high amount of squalene (3471.8µg/g). These results agree with Owen *et al.,* [23], they studied thirty oils comprising 18 virgin olive oil, 7 refined olive oil and 5 seed oils from Italian market and they found that squalene content in range of 3000 mg/kg in olive and seed oils. Meanwhile, when CEVOO is mixed with other two vegetable oils, squalene content decreases proportionally according to the ratio of mixed oils (Table3). It was noticeable that higher contents in campesterol and stigmasterol in SB (17.4, 14.0%) and SNF (10.4, 7.7%) oils compared to that found in CEVOO (0.7, 0.2%) Table 2. It is well known that in EVOO, campesterol, does not exceed 4.0% while, stigmasterol, always remains less than campesterol as set by Commission Regulation (EEC) No 2568/1991 [24]. Therefore, campesterol and stigmasterol could be used both as indicator to detect the adulteration of olive oil with these vegetable oils. 7- Stigmasterol and 7-avenasterol, which are not found in CEVOO and SBO, but only present in amount of 136.7 and 35.6 µg/g in SNF respectively.

The presence of Δ-7-stigmasterol and 7-avenasterol in olive oil is marked proof that olive oil is mixed with SNF at levels as low as 5%.These results agree with those reported by Bohacenko and Kopicova[25], who found that the contents of campesterol and Δ7-stigamsterol could be used to show the adulteration of olive oil at levels as low as 5%.

High ratio β-sitosterol/campesterol is specific to CEVOO (14.75%) while this ratio is low for SBO and SNF (3.09 and 4.79% respectively). Adulteration of CEVOO with SBO or SNF determined the decrease of this ratio (Table 3).These results agree with Dulf *et al*., [26] who reported that the high ratio β-sitosterol/campesterol is specific to virgin olive oil.

In an attempt to differentiate between the pure oils and their admixtures, the R ratio [β-sitosterol/ (campesterol+5-stigmasterol] (Imai *et al.,* [27]) was included where it amounted to 11.09 in CEVOO. It was observed that R ratio decreased to 8.0, 8.1 and 8.8 in case of SBO addition at 5, 10 and 20%. However, it reached to 8.9 and 7.4 when SNF mixed with CEVOO at 10 and 20 % (Table 3). Salvador *et al.,* [28] found that Cornicaba olive oil contains campesterol higher than 4% set by EC Regulation[24]. Therefore, Al-Ismail *et al.,* [29] reported that both campesterol and stigmasterol percentages can be taken to detect adulteration of olive oil with these oils by measuring the authenticity factor (Af) as follows:

Af= [100-(campesterol %+stigmasterol %)]/(campesterol %+stigmasterol %).

The Af factors of CEVOO was 1.34 and negative values of -0.7, -0.65 for SB and SNF oils respectively as shown in Table 2. The addition of SBO or SNF to CEVOO caused a decrease in Af factor (Table 3). These results demonstrated that Af factor is a valid parameter that could be used to detect adulteration of CEVOO, even its content of campesterol and stigmasterol are 0.7% and 0.2% respectively. Concerning the R ratio and Af

factor, it could be noticed that each pure oil had its own characteristic ratio. On the other side, these ratios varied in the admixtures and their values depending on the mixing ratio.

It is conclusively evident that Af factor and R ratio can indicate precisely the presence of adulteration of olive oil. These results demonstrated that Af factor and R ratio could be used to prove markedly adulteration of CEVOO. These findings are in harmony with those obtained (Salvador *et al*., [28,] EL-Shami *et al.*, [30]).

**Table 2: phytosterols and phytostanols of pure oils**

| **Phytosterols** | **Samples of oils µg/g** | | | | | |
|---|---|---|---|---|---|---|
| | **CEVOO** | | **SBO** | | **SNF** | |
| **Squalene** | **3471.8**±36.11 | **81**% | - | - | - | - |
| **Campesterol** | **32.0** ±4.8 | **0.7**% | **184.7**±9.8 | **17.4**% | **164.8**±4.8 | **10.4**% |
| **Campestanol** | - | - | **12.13**±4.2 | - | - | - |
| **Δ5-Stigmasterol** | **10.6** ±0.6 | **0.2%** | **148.9**±8.5 | **14.0%** | **121.4** ±3.8 | **7.7%** |
| **β-Sitosterol** | **472.8** ±24.6 | **11.0%** | **572.2**±32.1 | **53.9%** | **789.6** ±11.2 | **49.9%** |
| **Sitostanol** | - | - | **47.3**±1.7 | - | **139.1** ±1.3 | - |
| **Δ5-Avenasterol** | **101.0** ±16.5 | **2.4%** | - | - | **5.9**±0.6 | **0.37** |
| **Gramisterol** | **48.9** ±7.3 | - | - | - | - | - |
| **Cycloartenol** | **85.2**±5.8 | - | **83.6**±7.3 | - | **94.7**±5.3 | - |
| **Δ7-Stigmasterol** | - | - | - | - | **136.7** ±3.6 | **8.7%** |
| **Δ7-Avenasterol** | - | - | - | - | **35.6**±1.8 | **2.3%** |
| **24Methylene-cycloartenol** | **58.4**±1.4 | | **12.6** ±0.5 | - | **27.4**±2.1 | - |
| **Citrostadienol** | - | - | - | - | **64.6**±2.8 | - |
| **Total** | **4280.7** | - | **1061.52** | - | **1579.8** | - |
| **Ratios:** | | | | | | |
| **β-Sitosterol/ Campesterol** | **14.75** | - | **3.09** | | **4.79** | - |
| **R-ratio** | **11.09** | - | **1.72** | - | **2.75** | - |
| **Af factor** | **1.34** | - | **-0.7** | - | **-0.65** | - |

CEVOO: Coratina extra virgin olive oil, SBO: soybean oil, SNF: sunflower oil.

Af= [100-(campesterol %+stigmasterol %)]/( campesterol %+stigmasterol %).

R-ratio: β-Sitosterol / campesterol+ stigmasterol.

**Table 3: Phytosterols and phytostanols of simulated adulteration of the CEVOO with SBO and SNF**

| Phytosterols | Samples of simulated adulteration of the CEVOO with SBO and SNF (µg/g) | | | | | |
|---|---|---|---|---|---|---|
| | SBO in CEVOO wt % | | | SNF in CEVOO wt % | | |
| | 5% | 10% | 20% | 5% | 10% | 20% |
| **Squalene** | **3286.2** ±33.71 | **3102.6** ±30.92 | **2688.4** ±27.47 | **3263.1** ±31.33 | **3106.9** ±32.93 | **2967.2** ±27.98 |
| **Campesterol** | **40.0** ±0.90 | **46.3** ±1.11 | **59.3** ±1.5 | **37.8** ±0.8 | **43.5** ±1.1 | **56.6** ±1.4 |
| **Δ5-Stigmasterol** | **16.8** ±0.3 | **22.9** ±0.6 | **37.4** ±0.7 | **15.6** ±0.4 | **20.3** ±0.4 | **32.1** ±0.7 |
| **β-Sitosterol** | **470.6** ±6.3 | **483.8** ±5.9 | **491.3** ±6.5 | **485.4** ±6.4 | **503.2** ±6.3 | **538.7** ±7.2 |
| **Sitostanol** | **2.4** ±0.1 | **4.6** ±0.1 | **9.1** ±0.2 | **7.0** ±0.2 | **14.1** ±0.3 | **27.2** ±0.3 |
| **Δ5-Avenasterol** | **93.9** ±1.9 | **91.1** ±1.7 | **79.3** ±1.7 | **94.6** ±2.0 | **92.4** ±1.9 | **81.7** ±1.5 |
| **Cycloartenol** | **7.9** ±0.2 | **16.2** ±0.3 | **31.3** ±0.5 | **8.9** ±0.2 | **17.1** ±0.4 | **36.3** ±0.7 |
| **Δ7-Stigmasterol** | - | - | - | 6.7 ±0.1 | 13.6 ±0.2 | 26.9 ±0.2 |
| **24Methylene-cycloartenol** | **56.3** ±1.5 | **53.7** ±1.4 | **48.8** ±1.4 | **57.2** ±1.5 | **55.1** ±1.3 | **51.3** ±1.3 |
| **Δ7-avenasterol** | - | - | - | **2.0** ±0.1 | **3.4** ±0.1 | **7.2** ±0.2 |
| **Total** | **3974.1** | **3820.9** | **3744.9** | **3978.3** | **3869.6** | **3825.2** |
| **Ratios:** | | | | | | |
| **β-Sitosterol/ Campesterol** | **11.76** | **10.44** | **8.28** | **12.84** | **11.56** | **9.5** |
| **R-ratio** | **8.28** | **6.99** | **5.08** | **9.08** | **7.88** | **6.07** |
| **Af** | **0.67** | **0.44** | **0.035** | **0.87** | **0.56** | **0.12** |

CEVOO: Coratina extra virgin olive oil, SBO: soybean oil, SNF: sunflower oil,
R-ratio: β-Sitosterol / campesterol+ stigmasterol,
Af= [100-(campesterol%+stigmasterol %)] / (campesterol%+stigmasterol %).

Beside phytosterols evaluation, fatty acid compositions (FA) are important to characterize quality and specificity of oil. Olive oil is nutritionally considered one of the best salad vegetable oil due to the highest monounsaturated fatty acid content (MUFA), which is mainly due to the predominant presence of oleic acid (C18:1) and low saturated fatty acid content (Jiang *et al.*, [1] , Abdel-Razek *et a*l.,[2]). The content of MUFA and saturated fatty acid in CEVOO were 73.3 and 16.5% respectively, these results agree with Benincasa *et al.*, [9]. The content of MUFA in CEVOO was 73.3 and it decreases to 69.8, 66.2, 64.2as well as to 71.0, 69.5, 64.7 when CEVOO is mixed with SBO or SNF at ratio 5, 10 and 20% respectively (Table 4). On the other hand, the linoleic acid content of CEVOO constitutes 9.6% while in SBO and SNF reaches 54.8 and 58.7% respectively. Adulterated monovarietal CEVOO with SBO or SNF linoleic acid becomes higher than that of original CEVOO. Also, it was found that relative higher level of linolenic acid in CEVOO with SBO may prove that CEVOO is fraud with SBO even at low level (5%).

**Table 4: Fatty acid composition of pure oils and simulated adulteration CEVOO**

| Fatty acid | Pure oils | | | Simulated adulteration of the CEVOO with SBO and SNF | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | CEVOO | SBO | SNF | SBO in CEVOO wt % | | | SNF in CEVOO wt % | | |
| | | | | 5% | 10% | 20% | 5% | 10% | 20% |
| **C16:0** | **14.0**±0.6 | **10.5**±0.4 | **7.0**±0.2 | **14.5**±0.6 | **14.0**±0.6 | **12.4**±0.5 | **12.2**±0.5 | **11.4**±0.4 | **10.1**±0.3 |
| **C16:1** | - | **0.2**±0.1 | - | - | - | - | - | - | - |
| **C18:0** | **2.5**±0.1 | **3.3**±0.1 | **2.8**±0.1 | **1.1**±0.1 | **1.6**±0.2 | **2.1**±0.1 | **2.3**±0.1 | **2.1**±0.1 | **2.0**±0.1 |
| **C18:1 n-9** | **73.3**±3.1 | **25.7**±1.2 | **31.2**±1.3 | **69.0**±2.7 | **66.0**±2.6 | **63.3**±2.3 | **70.6**±2.6 | **68.6**±2.6 | **64.4**±2.4 |
| **C18:2 n-6** | **9.6**±0.3 | **53.8**±2.5 | **58.7**±2.6 | **14.3**±0.5 | **16.6**±0.5 | **20.0**±0.7 | **14.4**±0.5 | **17.5**±0.6 | **23.2**±0.6 |
| **C18:3 n-3** | **0.6**±0.1 | **5.8**±0.3 | **0.3**±0.1 | **1.1**±0.1 | **1.8**±0.1 | **2.2**±0.1 | **0.5**±0.1 | **0.4**±0.1 | **0.3**±0.1 |
| **C20:0** | - | **0.2**±0.1 | - | - | | - | - | - | - |
| **C20:1** | - | **0.5**±0.1 | - | - | - | - | - | - | - |
| **SFA** | **16.5** | **14.0** | **9.8** | **15.6** | **15.6** | **14.5** | **14.5** | **13.5** | **12.1** |
| **MUFA** | **73.3** | **26.4** | **31.2** | **69.0** | **66.0** | **63.3** | **70.5** | **68.6** | **64.4** |
| **UFA** | **83.5** | **86.0** | **90.2** | **84.4** | **84.4** | **85.5** | **85.4** | **86.5** | **87.9** |
| **PUSFA** | **10.2** | **59.6** | **59.0** | **15.4** | **18.4** | **22.20** | **14.9** | **17.9** | **23.5** |
| **Ratios:** | | | | | | | | | |
| **UFA/SFA** | **5.06** | **6.14** | **9.2** | **5.4** | **5.4** | **5.89** | **5.9** | **6.4** | **7.3** |
| **PUFA/SF** | **0.62** | **4.25** | **6.0** | **0.93** | **1.1** | **1.53** | **1.02** | **1.32** | **1.94** |
| **L/Ln** | **16** | **9.27** | **195.66** | **13.0** | **9.22** | **9.09** | **28.8** | **43.75** | **77.33** |
| **L/O** | **0.1** | **2.09** | **1.9** | **0.2** | **0.3** | **0.32** | **0.2** | **0.26** | **0.36** |
| **Ln/O** | **0.008** | **0.22** | **0.009** | **0.01** | **0.03** | **0.03** | **0.001** | **0.005** | **0.004** |
| **Cox value** | **1.85** | **6.87** | **6.42** | **2.4** | **2.76** | **3.07** | **2.29** | **2.57** | **3.09** |

In Table 4, it was found that variation in the values of ratio PUFA/SFA in adulterated oils with SBO or SNF different from that of CEVOO. Thus the ratio PUFA/SFA could be generally used in differentiating between pure and mixed oils. This value could be taken as an important parameter for determination of nutritional value of certain oil. Also, Cox values (measure of tendency of oils to undergo oxidation) proves the adulteration of CEVOO especially in 10 and 20% addition of SBO and SNF oils (Fatemi and Hammond [19], Mendez *et al.,* [31]). Olive oil contains more oleic acid and less linoleic and linolenic acids than other vegetable oils (Abdel-Razek *et al.,* [2]). Therefore, the ratio of linoleic and linolenic acid to oleic acid in olive oil can be used as a way to detect its adulteration with soybean oil and sunflower (Jakab *et al.,* [32,] Fasciotti and Annibal [33]). The data recorded in Table 4 shows that the ratio of linoleic acid to oleic acid (L/O) and linolenic acid to oleic acid (Ln/O) were 0.13 and 0.008 in CEVOO and slight changes in these ratios when EVOO mixed with SBO or SNF. With reference to ratio of linoleic to linolenic acid L/Ln, it was found that noticeable variation in the values of adulterated oils than that in CEVOO. In addition, marked changes in the ratio of USFA/ SFA especially in case of CEVOO mixed with 10 and 20% of SNF. Therefore, fatty acid composition and their ratios could be taken as useful means to determine the authenticity of CEVOO.

## 4. **Conclusions**

The obtained results confirm that the tocopherols, phytosterols, fatty acids, and some relations may find the type of vegetable oil added to the monovarietal CEVOO. The presence of Δ-7-stigmasterol and 7-avenasterol in CEVOO is a marked proof that olive oil is mixed with SNF at levels as low as 5%.Concerning tocopherol component, the detection of δ- tocopherols in the mixture confirmed the presence of SBO as foreign oil in CEVOO. In addition, the ratios α-/ γ-tocopherol and PUFA/ α-tocopherol were proposed for determining oil purity. In addition, ratio linoleic and linolenic acid to oleic acid in olive oil can be used to detect its adulteration with SBO or SNF even present in little amounts (5%).The analyses and relationships, mentioned above helps a lot to shed light on the characteristics of Egyptian monovarietal CEVOO as well as the detection of its adulteration.

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## **References**

1. Jiang L., Zhen H. and Lu H. Application of UV spectrometry and chemometric models for detecting olive oil-vegetable oil blends adulteration. J of Food Science and Technology, 2015, 52 (1) 479-485.
2. Abdel-Razek A. G., El-Shami S. M., El-Mallah M. H. and Hassanein M. M. (2011). Blending of virgin olive oil with less stable edible oils to strengthen their antioxidative potencies. Australian Journal of Basic and Applied Sciences, 5 (10): 312-318.
3. De Nino, A., Mazzotti F., Perri E., Procopio A., Raffaelli A. and Sindona G**.** Virtual freezing of hemiacetal-aldehyde equilibrium of the aglycones of oleuropein and ligstroside present in olive oils from Carolea and Coratina cultivars by ion spray ionization tandem mass spectrometry. J. Mass Spectrum, 2000, 35,461-467.
4. Angerosa F., Influence of volatile compounds on virgin olive oil quality evaluated by analytical approaches and sensory panels. Eur. J. Lipid Sci. Tech., 2002,104, 639-660.
5. Duran R.M., Relationship between the composition and ripening of the olive and the quality of the oil. Acta Horticulture, 1990, 286, 441-452.
6. Babcock, B. A. and Clemens R. Geographical indications and property rights: Protecting value-added agricultural products. MATRIC Briefing Paper 04-MBP 7, Iowa State University, Ames, IA (USA) 2004.
7. Ozen B., Tokatli F. and Korel F. Emerging topics in olive oil research: Determination of geographical origin and adulteration. Olive Oil and Olive-Pomace Oil Symposium & Exhibition Izmir (Turkey) 2005.
8. Abd-Alhamid, N. , Laila F. Haggag, H.S.A. Hassan, A. A. Abdelhafez and A. M. Hassan. Effect of mineral and bio-fertilization on yield and fruit quality of Manzanillo Olive trees. International Journal of Chem. Tech. Research, 2015, 8 (11), 63-73.
9. Benincasa C., Russo A., Romano E., Elsorady M. E., Perri E. and MuzzalupoI. Chemical and sensory analysis of some Egyptian virgin olive oils. J. Nutr. Food Sci., 2011, 1, 1-5.
10. Christopoulou E.,Lazaraki M., Komaitis M.and Kaselimis K., Effectiveness of determinations of fatty acids and triglycerides for the detection of adulteration of olive oils with vegetable oils. Food Chem., 2004, 84,463–474.
11. Cercaci L., Rodriguez-Estrada M. T. and Lercker G. Solid-phase extraction- thin layer chromatography–gas chromatography method for the detection of hazelnut oil in olive oils by determination of esterified sterols. J. Chromatogr. A, 2003,985, 211–220.
12. Oliveros M. C. C.,PavónJ. L. P., Pinto C. G., Laespada M. E. F., Cordero B. M. and Forina M. Electronic nose based on metal oxide semiconductor sensors as a fast alternative for the detection of adulteration of virgin olive oils. Anal. Chim. Acta., 2002,459,219–228.
13. Pena, F., Cárdenas S., Gallego M. and Valcárcel M., Direct olive oil authentication: Detection of adulteration of olive oil with hazelnut oil by direct coupling of headspace and mass spectrometry, and multivariate regression techniques. J. Chromatogr. A, 2005, 1/2, 215–221.

14. Poulli, K. I., Mousdis G. A. and Georgiou C. A., Rapid synchronous fluorescence method for virgin olive oil adulteration assessment. Food Chem., 2007, 105 (1), 369–375.
15. Balz, M., Schulte E. and Their H. P. Trennung von Tocopherolen und Tocotrienolendurch HPLC European. J. Lipid Sci. Technol., 1992, 94,209–213.
16. Hassanien M. M.M., Abdel-Razek A. G., Rudzińska M., Siger A., Ratusz K.and Przybylski R. Phytochemical contents and oxidative stability of oils from non-traditional sources. Eur. J. Lipid Sci. Technol., 2014, 116, 1563–1571.
17. AOCS. Official Method Ch 6–91, Determination of the Composition of the Sterol Fraction of Animal and Vegetable Oils and Fats by TLC and Capillary GLC. Official Methods and Recommended Practices of the American Oil Chemists' Society, 4th ed. 1997, American Oil Chemists' Society, USA.
18. AOCS, Official Method Ce 1k-07, Direct Methylation of Lipids for the Determination of Total Fat, Saturated, *cis*-Monounsaturated, *cis*-Polyunsaturated, and *trans* Fatty Acids by Chromatography. Official Method and Recommended Practices of the American Oil Chemists' Society, 5th ed. 2007, American Oil Chemists Society, USA.
19. Fatemi, S. H. and Hammond E. G., Analysis of oleate, linoleate and linolenate hydroperoxides in oxidized ester mixtures. Lipids, 1980, 15,379–385.
20. Rossell J. B., King B. and Downes M. J. Detection of adulteration. J. Am. Oil Chem. Soc., 1983, 60 (2) 333-339.
21. Alfin-Slater R. B., Wells P. and After good L., Dietary fat composition and tocopherol requirement: IV Safety of polyunsaturated fat. J. Am. Oil Chem. Soc., 1973, 50 (12) 479-484.
22. Deiana M, Rosa A., Cao C. F., Pirisi F. M., Bandino G. and Dessi M. A. Novel approach to study oxidative stability of extra virgin olive oils: importance of α-tocopherol concentration. J Agric. Food Chem., 2002, 50 (15) 4342–4346.
23. Owen R. W., Mier W., Giacosa W., Hull E., Spiegelhalder B. and Bartsch H. Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of totalphenols, simple phenols, secoiridoids, lignans and squalene. Food and Chemical Toxicology, 2000, 38,647-659.
24. EC Regulation 2568. The characteristics of olive oil and olive residue oil and relevant methods of analysis. Official Journal L., 1991, 248, 6-7.
25. Bohacenko, I. and Kopicova Z. Detection of olive oils authenticity by determination of their sterol content using LC/GC. Czech Journal of Food Science, 2001, 19, 97-103.
26. Dulf, F.V., Bele C, Ungureşan M.L. and Socaciu C. Qualitative and quantitative markers to identify the quality and adulteration of olive oil with rapeseed oil. Buletin USAMV-CN, 2007, 575-580.
27. Imai C., Watanabe H. N., Haga N., and T.II, Detection of adulteration of cottonseed oil by gas chromatography. J. Am. Oil Chem. Soc. 1974, 51 (7) 326-330.
28. Salvador M. D., Aranda F. and Fregapane G. Chemical composition of commercial Cornicabra virgin olive oil from 1995/96 and 1996/97 Crops. J. Am. Oil Chem. Society, 1998, 75, 1305-1311
29. Al-Ismail K. M., Alsaed A. K., Ahmad R., Al-Dabbas M. Detection of olive oil adulteration with some plant oils by GLC analysis of sterols using polar column. Food Chem., 2010,121, 1255−1259.
30. EL-Shami, S. M. Determination of fatty acids and sterols in some vegetable oils by capillary gas chromatography. J. Japan. Oil Chem. Soc. (YUKAGAKU), 1992, 41(10) 1067-1070.
31. Mendez, E., J. Sanhueza, H. Speisky and A. Valenzuela. Validation of the rancimat test for the assessment of the relative stability of fish oils. J. Am. Oil Chem. Soc., 1996, 73: 1033-1037.
32. Jakab A., Heberger K., Esther Forgacs, Comparative analysis of different plant oils by high-performance liquid chromatography-atmospheric ionization mass spectrometry, J. Chrom. A, 2002, 976, 255-263.
33. Fasciotti M., Annibal D. P. N., Optimization and application of methods of triacylglycerol evaluation for characterization of olive oil adulteration by soyabean oil with HPLC-APCI-MS-MS. Talanta, 2010, 81, 1116-1125.

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