

Detection of argan oil adulterated with vegetable oils: New markers

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RESUMEN

Detección de aceite argán adulterado con aceites vegetales: Nuevos marcadores.

El objetivo principal de este trabajo es contribuir al control de la autenticidad del aceite argán, un producto marroquí muy valorado. Con el fin de detectar la adulteración del aceite de argán con aceites vegetales comestibles, se han estudiado los siguientes parámetros: ácidos grasos, fracción de hidrocarburos, 3,5-estigmastadieno, ésteres alquílicos, pigmentos clorofílicos y propiedades físicas como la viscosidad, densidad e índice de refracción. Los resultados de este estudio muestran que el 3,5-estigmastadieno, kaureno y la feofitina-a podrían utilizarse como nuevos marcadores en la detección del aceite de argán adulterado con aceites refinados y aceite oliva virgen al 5%. La composición en ácidos grasos puede emplearse para la detección de la adulteración del aceite de argán a niveles superiores al 10%, debido a la similitud en la composición de los aceites estudiados. Entre las propiedades físicas analizadas, el índice de refracción mostró diferencias significativas entre el aceite de argán y sus mezclas con el aceite de girasol al 10%.

PALABRAS CLAVE: Aceite de argán – Aceite de girasol – Aceite de oliva – Adulteración – GC – HPLC.

SUMMARY

Detection of argan oil adulterated with vegetable oils: new markers.

This work aims to contribute to controlling the authenticity of pure argan oil, a valuable Moroccan product. Fatty acids, hydrocarbon fraction, 3,5-stigmastadiene, the alkyl esters of fatty acids, chlorophyllic pigments and physical properties such as viscosity, density and refractive index were studied in order to detect the adulteration of argan oil with edible vegetable oils. The results found in this study show that 3,5-stigmastadiene, kaurene and pheophytin-a can be used as possible new markers for argan oil blends of up to 5% with refined, sunflower and virgin olive oils. Due to the similarity of the fatty acid compositions of the edible oils studied and argan oil, fatty acids can be employed as markers for the detection of argan oil adulteration at levels higher than 10%. Among the physical properties studied, the refractive index shows significant differences for sunflower oil and its blend at 10% with argan oil.

KEY-WORDS: Adulteration – Argan oil – GC – HPLC – Olive oil – Sunflower oil.

1. INTRODUCTION

Argan oil is obtained from the fruits of the argan tree (*Argania spinosa*) which is an endemic species of south-western Morocco, where it plays a major ecological and socio-economic role (Khallouki *et al.*, 2003). To protect this unique eco-system, UNESCO declared the Arganeraie a Biosphere Reserve in 1998 (Lybbert *et al.*, 2002). This plant is well adapted to semiarid conditions that exhibit physiological and morphological adjustments in response to drought. However, in a scenario of global change and increase in drought in arid and semi-arid areas, its distribution area is likely to be reduced to milder areas such as the coastal fringe, and it will also be more sensitive to human pressure such as water abstraction and browsing (Díaz-Barradas *et al.*, 2010).

Argan oil is well known for its bactericidal and fungicidal properties as well as other beneficial effects on health. Moroccan folk medicine uses argan oil based preparations for their hepatoprotective, hypocholesterolemic, and anti-atherosclerotic effects, as well as in the treatment of different diseases such as rheumatism, and dermatological or articular pathologies (Moukal 2004; Charrouf and Guillaume 2008). The chemical composition of this oil has been studied and it was shown that the geographical origin of the argan fruit, roasting time and extraction method used to produce the oil have considerable influence on its physico-chemical composition and characteristics (Hilali *et al.*, 2005, Cayuela *et al.*, 2008, Marfil *et al.*, 2008, Harhar *et al.*, 2011; Guinda *et al.*, 2011). A Moroccan norm was created in 2003 to define the quality specifications of virgin argan oil and its classification into different categories; extra virgin argan oil is the highest quality level (Norme Marocaine 2003). Four thousand tons of argan oil are produced in Morocco every year, of which ca. 2500 tons are destined to exportation. The price of argan oil in Europe is approximately 100 euros per liter and is considered a luxury food. Due to its price there is always the possibility that illegal practices will occur such as dilution with cheaper oils (olive oil, colored with paprika or other substances, González *et al.*, 2010). The detection of oil adulteration is a complex problem. Indeed, the

mixture of two oils of similar composition can be hard to detect as evidenced in olive oil adulteration with hazelnut oil (Christy *et al.*, 2004; Zabarás 2010). At present, studies that discriminate argan oil from other edible oils are scarce. In recent years, González *et al.*, (2010) established the trace element profile of argan oil by means of inductively coupled plasma optical emission measurements in combination with different chemometric approaches. The studies were performed using 16 variables (Na, Mg, Al, K, Ca, Ti, Fe, Co, Ni, Cu, Zn, Cd, Pr, Sm, Er and Bi). Hierarchical cluster analysis is able to differentiate sunflower oil samples from the rest, however the discrimination of argan oil from olive, seed and soy oils based on their different trace element compositions is hard to achieve. Režanka and Režanková 1999, considered cluster analysis and multidimensional scaling with chi-square measurements of dissimilarity the best methods for the determination of similarity of oils based on percentage contents, fatty acids or triacylglycerols. Analytical detection of possible argan oil adulteration is much easier and shorter when multivariate statistical methods are used. Hilali *et al.*, (2007) proposed campesterol to be an adulteration marker because of its determination level in argan oil, and suggested that it could be the major analysis factor to assess oil purity up to 98%. Campesterol rich oils, (soybean, rapeseed, arachis, sesame) were detected at adulteration levels of 1%. Similar results were obtained with sunflower oil. For the low campesterol containing oils, this method allows for the establishment of a 95% purity label in the case of olive and hazelnut oil and 98% purity label for apricot oil. In addition, ferulic acid has been confirmed as a phenolic compound marker to control the authenticity of argan oil (Zougagh *et al.*, 2011). The authors developed a method based on the formation of gold nanoparticles and spectrophotometric analysis to evaluate the adulteration of virgin argan oils.

In order to detect the adulteration of argan oil with edible vegetable oils, the parameters taken into account in this study include 3,5-stigmastadiene, fatty acids alkyl esters, chlorophyllic pigments and hydrocarbons.

Several studies on the characterization of edible vegetable oils by analyzing the hydrocarbon fraction have been reported. Hydrocarbons are the least polar compounds of the unsaponifiable matter of vegetable oils (Moreda *et al.*, 2001). The determination of hydrocarbons by gas chromatography has been used both in the characterization of edible vegetable oils, especially in virgin olive oils; as well as in the fraudulent adulteration of virgin olive oils with cheaper oils. (Lanzón *et al.*, 1989, Webster *et al.*, 2000, Bueno *et al.*, 2005, Guinda *et al.*, 1996). On the other hand, a significant amount of a steroidal compound, 3,5-stigmastadiene, is formed in vegetable oils due to thermal treatment during the refining processes (Lanzón *et al.*, 1994). This compound is derived from the dehydration of β -sitosterol (Cert *et al.*,

1994). Depending on the refining conditions, refined vegetable oils showed 3,5-stigmastadiene contents between 1 and 29 mg kg⁻¹ (Dobarganes *et al.*, 1999); therefore, the assessment of stigmastadiene in vegetable oils allows not only for the identification of thermally treated oils but also the detection of virgin olive oil adulteration with refined oils. The alkyl esters of fatty acids are a family of natural neutral lipids present in olive oil and formed by the esterification of free fatty acids with low molecular alcohols. Inappropriate practices during the olive oil extraction process and a low quality olive fruit promote their formation. Blends of extra virgin olive and "soft" refined low quality olive oils can be detected by their alkyl ester concentrations (Pérez-Camino *et al.*, 2002). The composition of fatty acid alkyl esters in argan oil have not been studied before, however in olive oil, these compounds have been widely studied (Ruiz-Méndez *et al.*, 2003, Pérez-Camino *et al.*, 2008). Therefore, fatty acid alkyl ester analysis was considered interesting to detect the presence of low quality virgin olive oil in argan oil. In this work, chlorophyllic pigments are analyzed in order to detect possible argan oil blends with extra virgin olive oil. This oil presents the combination of green and yellow colors due to the presence of chlorophyll and carotenoid pigments (Gandul-Rojas *et al.*, 2000) which are not present in seed oils. In addition, we considered the study of viscosity in oils interesting for the possible detection of virgin argan oil adulteration, as this physical parameter is directly related to the triacylglycerol composition, the major component of oils. Furthermore, this determination is direct and economical. Finally, we have analyzed other physical properties directly related to the purity of oils such as their density and refractive index.

2. MATERIALS AND METHODS

2.1. Material

Oils samples

Sixty percent of the argan oil samples were purchased in a Moroccan market and the rest of the argan oil samples were supplied by the industrial plant Arganoil Company Ltd. The sunflower and olive oils were purchased in a Spanish market. All samples were kept at -40°C until analysis. For identification of the oil blends, see Table 1. Ninety-six oil samples were used to carry out this study.

Reagents and standards

All reagents were of analytical reagent grade unless otherwise stated. Standards of n-eicosane, 3,5-cholestadiene, methyl heptadecanoate and chlorophyll-a were purchased from Sigma-Aldrich (Sigma-Aldrich Química, Madrid, Spain). The SPE cartridge (6 mL), packed with silica gel phase (1 g), was from Varian (EA Middelburg, The Netherlands).

Table 1
Argan blend oil samples

| Blend oils | | | | |
|------------|----------|----------|----------|----------|
| (%) | VOO | OO | HOSO | SO |
| 50 | Blend 1 | Blend 2 | Blend 3 | Blend 4 |
| 10 | Blend 5 | Blend 6 | Blend 7 | Blend 8 |
| 5 | Blend 9 | Blend 10 | Blend 11 | Blend 12 |
| 3 | – | Blend 13 | Blend 14 | Blend 15 |
| 1 | Blend 16 | Blend 17 | Blend 18 | Blend 19 |

VOO: Virgin Olive Oil; OO: Olive Oil; HOSO: High Oleic Sunflower Oil; SO: Sunflower Oil.

2.2. Fatty acid composition

The fatty acid composition of the oil samples is determined by gas chromatography as fatty acid methyl esters. The Trans-esterification of triacylglycerides is carried out using a cold methanolic solution of potassium hydroxide, according to the European Union Commission 1991. For the chromatographic determination of fatty acid methyl esters an HP 6890N gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a capillary column (Supelcowax: 60 m × 0.25 mm; 0.25 mm), automatic split injector and a flame ionization detector (FID) was used. The carrier gas was hydrogen, with a flow rate of 1 mL min⁻¹. The temperatures of the injector and detector were held at 210 and 250 °C, respectively. The oven was programmed at 165 °C (10 min) and increased by 1,5 °C min⁻¹ up to 200 °C (10 min). The injection volume was 1 µL. The fatty acid composition is expressed as relative percentage of each fatty acid.

2.3. Determination of physical properties

Traditionally, density is determined using a 25 mL picnometer previously calibrated with distilled water and the dynamic viscosity measurement is determined with a temperature controlled falling ball viscometer or with a rheometer equipped with a coneplate, according to the standards. In this work, a Stabinger SVM 3000 (Anton Paar GmbH Graz, Austria) is employed for the viscosity and density determinations. The filling of the instrument and the cleaning are manual. The SVM is equipped with fast thermostating (no water bath required) and data measuring, which are carried over to a PC system via the serial interface. A volume of 5 mL of sample is employed. Each test is carried out three times. For the cell filling, plastic disposable syringes are used and n-hexane is employed for the cell cleaning. The resulting values are only accurate to the third and second digit after the comma, for density and viscosity, respectively. Refractive indices were determined with a temperature-controlled Abbe refractometer (Hilger & Watts Ltd., London, U.K.), using white light for the measurement (IUPAC, 1992,

No. 2102). Each of these three physical parameters was determined at 20 and 40 °C in all vegetable oils in the study.

2.4. Hydrocarbon fraction

Linear hydrocarbon quantification

The isolation of the hydrocarbon fraction is performed by means of the alkaline hydrolysis of 20 g of the oil sample spiked with 0.25 mL of a standard solution of n-eicosane (0.05 mg mL⁻¹), as described by Guinda *et al.*, 1996. The unsaponifiable residue is fractionated by means of a silica gel low-pressure column (15 g) using hexane as eluent. The fraction collected by eluting with 35 mL is concentrated to approximately 1 mL. An aliquot of 1 µL of this fraction, containing saturated and unsaturated aliphatic hydrocarbons, is analyzed by gas chromatography using an HP 6890N gas chromatograph equipped with a capillary column (SPB5: 10 m × 0.32 mm, Supelco, Bellefonte, PA), split injector and FID detector. The carrier gas is hydrogen with a flow rate of 1 mL min⁻¹. The operating conditions were as follows: initial oven temperature of 60 °C for 1 min and then programmed at 12 °C per min⁻¹ up to 280 °C for 1 min, followed by a 7 °C min⁻¹ increase up to 340 °C for 1 min. Injector and detector temperatures were 280 °C and 350 °C, respectively.

Steroidal hydrocarbons determination

The quantitative determination of 3,5 stigmastadiene in vegetable oils follows the methodology standardized by IUPAC (Dobarganes *et al.*, 1999). The oil sample (20 g), spiked with 1 mL of internal standard (3,5-cholestadiene; 20 µg mL⁻¹), is saponified and the unsaponifiable is fractionated in a silica gel low-pressure column using hexane as eluent. The first 35 mL of the eluate were discarded, the following 40 mL fraction is evaporated until dryness, and the residue is dissolved in 0.5 mL of heptane. An aliquot of 1 µL is analyzed by gas chromatography using an HP 6890 gas chromatograph equipped with a capillary column (SGL5: 30 m × 0.25 mm; 0.25 mm), split injector and FID detector. The carrier gas was hydrogen, with a flow rate of 1 mL min⁻¹. The temperatures of the injector and detector were held at 280 and 300 °C respectively. The oven was programmed at 210 °C for 5 min and then increased by 3 °C min⁻¹ up to 280 °C (6 min).

2.5. Alkyl ester quantification

Methyl and ethyl esters are determined according to the Pérez-Camino *et al.* (2002) procedure. Briefly, 0.2 g of the oil sample were dissolved in 1 mL of hexane and mixed with 500 µL of a standard solution of 0.1 mg mL⁻¹ methyl heptadecanoate. A 1g silica SPE cartridge fractionated one milliliter of this

solution. A mixture of hexane/toluene (85:15; v/v) was employed as eluent. The first 7 mL were discarded and the following 10 mL fraction was collected and evaporated under reduced pressure. The residue was dissolved in 300 μ L of heptane. The gas chromatographic determination of the alkyl esters was carried out using an HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a fused silica capillary column coated with cyanopropylsilicone (SPB5 10 m \times 0.32 mm, Supelco, Bellefonte, PA). Hydrogen was used as carrier gas. The initial oven temperature of 70 $^{\circ}$ C was increased by 15 $^{\circ}$ C min^{-1} up to 150 $^{\circ}$ C; then by 12 $^{\circ}$ C min^{-1} up to 340 $^{\circ}$ C and maintained for 12 min at the final temperature. The detector temperature was 350 $^{\circ}$ C. The injection volume was 1 μ L.

2.6. Pigment analysis

Separation of the pheophytins and pyropheophytin pigments was carried out by SPE, according to Hornero-Méndez *et al.*, (2005). After eluting with 12 mL petroleum ether/diethyl ether solvent mixture 90:10, the major part of the lipids was discarded and a fraction containing the pigments was collected after eluting with 3 mL acetone. The eluate was analyzed by HPLC (HP 1100; Hewlett-Packard, Palo Alto, CA) equipped with a stainless steel column (25 cm \times 0.46 cm i.d.), packed with 5 μ m C18 Spherisorb ODS-2 (Teknokroma, Barcelona, Spain). An isocratic elution with methanol-acetone-water (36:60:4, v/v/v) was used. Sequential detection was performed with a diode array detector. Pigments were quantified at the wavelength of maximum absorption (410 nm) with a chlorophyll calibration curve at a concentration range according to the levels of this pigment in virgin olive oil. For the purpose of this determination it was assumed that the response factors of all pigments were equal.

2.7. Statistical analysis of experimental data

ANOVA was carried out using the SigmaStat 3.5 statistical pack, (Systat Software Inc.) Each reported value is the mean of two measurements from four replicates. Dunnett's Method of multiple comparisons versus the control group (virgin argan oil) was applied to determine significance among means. Statistical significance was considered at $p < 0.05$.

3. RESULTS AND DISCUSSION

The chemical and physical characteristics of pure argan oil, olive and sunflower oils and their blends with argan oil are summarized in Tables 2, 3, 4, 5 and 6. Fatty acid methyl esters, viscosity, density, refractive index, stigmastadiene compound, hydrocarbon fraction, alkyl esters and chlorophyll pigments, were selected as possible

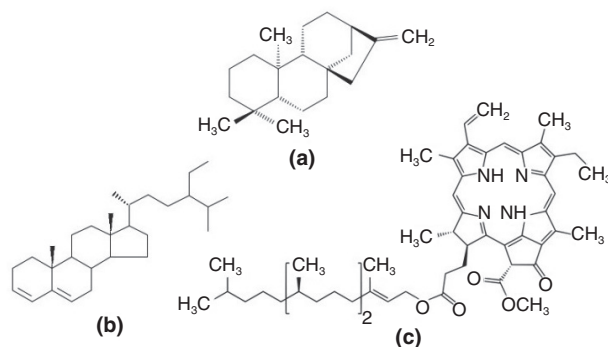


Figure 1
Molecular structures of: kaurene (a), stigmastadiene (b) and pheophytin a (c).

markers for the detection of argan oil contamination or adulteration.

3.1. Fatty acid methyl esters

Table 2 shows the results of the analysis of fatty acid methyl esters of argan, other vegetable oils (VOO, OO, HOSO and SO) and their blends with argan oil. The fatty acid compositions of the pure argan oil and the other vegetable oils were in accordance with those found in the literature (Hilali *et al.*, 2005, Cayuela *et al.*, 2008). The major fatty acids in VAO, HOSO and SO were oleic and linoleic acids. The VAO, HOSO and SO oils showed the following relative percentage of oleic and linoleic acids: 45.23 and 35.51%; 79.26 and 11.15%; 19.68 and 68.02%, respectively, whereas the major fatty acid present in VOO and OO, was oleic acid. The VOO and OO oils showed the following relative percentage of oleic acid: 73.62 and 76.42%, respectively. The results in Table 2, clearly reveal the fact that the major fatty acids could be used as markers for the detection of argan oil blends with other oils studied when the percentage is higher than 10% (statistically significant differences, $\alpha = 0.05$). The non-major fatty acid, palmitic acid, with a percentage lower than 12.65 in all the oils studied, only presented significant differences between pure argan oil and its sunflower oils blends at 10%. As shown in Table 2, the minority fatty acids cannot be used to detect any of these argan oil blends. Due to the similarity in fatty acid composition of the edible oils studied and argan oil, the fatty acids cannot be employed as markers for the detection of argan oil adulteration at levels lower than 10%.

3.2. Physical properties: viscosity, density and refractive index

Table 3 shows a selection of physical properties of pure argan oil olive and sunflower oils and their blends with argan oil. An average of sixteen samples (VOO and OO) was used for the study of olive oils since value ranges of these parameters are close to each other because this parameter is directly related with triacylglycerol composition.

Table 2
FAMES Percentage in pure and blended oils

| Sample [†] | C16:0 | SD | C16:1 | SD | C18:0 | SD | C18:1 | SD | C18:2 | SD | C20:0 | SD | C18:3 | SD | C20:1 | SD | C22:0 | SD |
|---------------------|--------|------|-------|------|-------|------|--------|------|--------|------|-------|------|-------|------|-------|------|-------|------|
| VAO | 12,65 | 0,25 | 0,10 | 0,01 | 5,73 | 0,29 | 45,23 | 0,77 | 35,51 | 0,89 | 0,35 | 0,02 | 0,26 | 0,03 | 0,35 | 0,02 | 0,11 | 0,01 |
| VOO | 12,04* | 0,24 | 0,74* | 0,05 | 3,25* | 0,20 | 73,62* | 1,06 | 8,53* | 0,34 | 0,29* | 0,03 | 0,8* | 0,05 | 0,28* | 0,03 | 0,16* | 0,02 |
| OO | 10,47* | 0,21 | 0,72* | 0,05 | 3,75* | 0,23 | 76,42* | 1,10 | 7,14* | 0,29 | 0,40* | 0,03 | 0,63* | 0,04 | 0,26* | 0,03 | 0,14* | 0,01 |
| HOSO | 3,68* | 0,22 | 0,10 | 0,01 | 4,30* | 0,26 | 79,26* | 1,14 | 11,15* | 0,22 | 0,21* | 0,02 | 0,21* | 0,02 | 0,08* | 0,01 | 0,7* | 0,04 |
| SO | 6,62* | 0,33 | 0,20* | 0,02 | 3,97* | 0,24 | 19,68* | 0,49 | 68,02* | 0,98 | 0,28* | 0,03 | 0,25 | 0,03 | 0,15* | 0,02 | 0,69* | 0,04 |
| Blend 5 | 12,41 | 0,32 | 0,13 | 0,01 | 5,65 | 0,28 | 49,03* | 0,84 | 32,09* | 0,80 | 0,36 | 0,02 | 0,27 | 0,03 | 0,36 | 0,02 | 0,1 | 0,01 |
| Blend 6 | 12,37 | 0,30 | 0,14 | 0,01 | 5,70 | 0,28 | 49,32* | 0,84 | 31,82* | 0,80 | 0,38 | 0,02 | 0,25 | 0,03 | 0,36 | 0,02 | 0,1 | 0,01 |
| Blend 7 | 11,52* | 0,23 | 0,10 | 0,01 | 5,59 | 0,27 | 49,36* | 0,85 | 32,64* | 0,82 | 0,35 | 0,02 | 0,27 | 0,03 | 0,26* | 0,03 | 0,19* | 0,02 |
| Blend 8 | 12,07* | 0,24 | 0,09 | 0,01 | 5,72 | 0,29 | 43,53* | 0,75 | 37,21* | 0,93 | 0,36 | 0,02 | 0,22 | 0,02 | 0,35 | 0,02 | 0,15* | 0,02 |

[†]mean, n = 8; *significant difference ($\alpha = 0,05$); C17:0 $\leq 0,1$ in all oils and blended samples. VAO: Virgin Argan Oil; VOO: Virgin Olive Oil; OO: Olive Oil; HOSO: High Oleic Sunflower Oil; SO: Sunflower Oil. See Table 1 for blend descriptions.

The viscosity, density and refractive index of VAO at 20 °C and 40 °C were 70.47 and 31.33 mPa.s, 0.9155 and 0.9019 g cm⁻³ and 1.4701 and 1.4631, respectively. The density and refractive index results are in accordance with the data found in the literature (Belarbi-Benmahdi *et al.*, 2009, Hilali

et al., 2005) and Moroccan regulation (Norme Marocaine. 2003).

The data published on the viscosity of argan oil, at 30 °C, by Yaghmur *et al.*, (2001), were consistent with the theoretical extrapolation of our data at the same temperature. In addition, the results on

Table 3
Physical properties of pure and blended oils

| Sample [†] | η (mPa . s) | SD | ρ (g cm ⁻³) | SD | nt | SD |
|----------------------|------------------|------|------------------------------|--------|---------|-------|
| 20 °C | | | | | | |
| VAO | 70,47 | 1,20 | 0,9155 | 0,0031 | 1,4701 | 0,001 |
| OO/VOO | 80,13* | 1,42 | 0,9123 | 0,0030 | 1,4683* | 0,001 |
| HOSO | 79,62* | 0,83 | 0,9127 | 0,0002 | 1,4695 | 0,001 |
| SO | 67,79* | 1,01 | 0,9187 | 0,0031 | 1,474* | 0,001 |
| Blend 1/2 | 75,38* | 1,50 | 0,9127 | 0,0020 | 1,4693 | 0,001 |
| Blend 3 | 74,70* | 1,60 | 0,9134 | 0,0025 | 1,4698 | 0,001 |
| Blend 4 | 68,97 | 1,00 | 0,9162 | 0,0026 | 1,4721* | 0,001 |
| Blend 5/6 | 70,60 | 0,98 | 0,9162 | 0,0032 | 1,4699 | 0,001 |
| Blend 7 | 71,68 | 1,40 | 0,9149 | 0,0002 | 1,47 | 0,001 |
| Blend 8 | 70,65 | 1,40 | 0,9151 | 0,0017 | 1,4705 | 0,001 |
| 40 °C | | | | | | |
| VAO | 31,33 | 0,89 | 0,9019 | 0,0024 | 1,4631 | 0,001 |
| OO/VOO ^{††} | 34,81* | 0,90 | 0,8987* | 0,0012 | 1,4613* | 0,001 |
| HOSO | 34,80* | 0,78 | 0,8991 | 0,0020 | 1,4625 | 0,001 |
| SO | 30,91 | 0,87 | 0,9060* | 0,0034 | 1,467* | 0,001 |
| Blend 5/6 | 31,79 | 0,95 | 0,8991 | 0,0021 | 1,4623 | 0,001 |
| Blend 7 | 32,00 | 0,88 | 0,8998 | 0,0030 | 1,4628 | 0,001 |
| Blend 8 | 31,34 | 1,03 | 0,9026 | 0,0011 | 1,4651* | 0,001 |

[†]mean, n = 8; ^{††}mean, n = 16; *significant difference ($\alpha = 0,05$); VAO: Virgin Argan Oil; VOO: Virgin Olive Oil; OO: Olive Oil; HOSO: High Oleic Sunflower Oil; SO: Sunflower Oil. See Table 1 for blend descriptions.

viscosity, density and refractive index of the oils employed to prepare the blends in this study are in accordance with the literature data. At 20 °C, the viscosity results only present differences between mixtures 50/50 of the olive oils and HOSO with the argan oil (blends 1/2 and 3). None of the studied oils or their mixtures presented significant differences in density values at 20 °C. Nevertheless, at the same temperature, the refractive index shows significant differences only for sunflower oil and its blend at 50% with argan oil (blend 4), because the values of the refractive index of olive and HOSO oils are close to each other. Although in Table 3 significant differences were observed in the values of the viscosity of pure VAO, HOSO and olive oils, at 40 °C, the argan blends 5/6 and 7 (10%) presented no differences ($\alpha = 0.05$). The viscosity determined in these conditions does not serve to detect blends of argan oil with the studied oils up to 10%. The density value of sunflower and olive oils at 40 °C, presented significant differences with argan oil but no significant differences were observed with their mixtures up to 10% (blends 5/6 and 8). At this temperature, the density does not serve as a parameter to detect blends of argan oil with oils studied up to 10%. The refractive index shows significant differences for sunflower oil and its blend at 10% with argan oil (blend 8) at 40 °C, as well as at 20 °C.

3.3. Hydrocarbons fraction

Table 4 shows the results of the hydrocarbon fraction analysis of argan oil, vegetable oils (VOO, OO, HOSO and SO) and their VAO blends. The comparison of hydrocarbon fraction composition between the VAO and the other vegetable oils revealed that in sunflower oils, the major hydrocarbons presented are C27, C29 and C31, however in olive oils C29 and C31 are the main hydrocarbons, according to the published data (Bortolomeazzi *et al.* 2001, Bueno *et al.* 2005). Figure 2 shows the gas chromatogram of the TLC fraction obtained in the capillary column. Peak 1, was identified as kaurene on the basis of its mass spectra, a diterpene hydrocarbon present in sunflower oils (HOSO and SO) which is absent in olive oils, either virgin or not. Furthermore, the most interesting data observed in Figure 2 is that virgin argan oil does not contain kaurene. Taking into account the results presented in Table 4, kaurene can be considered a possible marker of argan oil adulteration with sunflower oils up to 5%. In accordance with published data, Table 4 presented a kaurene content of 27.18-30.52 ppm and a not identified hydrocarbon content of 52.28-44.81 ppm (RT 12.8 min) in sunflower oils HOSO and SO, respectively. One-way analysis of variance of C25 showed no significant difference ($\alpha = 0.05$) between

Table 4
Hydrocarbon fractions of pure and argan blend oils

| Sample [†] | Hydrocarbon (mg kg ⁻¹) | | | | | |
|---------------------|------------------------------------|-------------------|--------------|---------------|---------------|---------------|
| | K | UP (RT = 12.8) | C25 | C27 | C29 | C31 |
| VAO | 0.00 | 0.00 | 0.11 ± 0.10 | 1.63 ± 0.23 | 2.09 ± 0.32 | 2.22 ± 0.34 |
| VOO | 0.00 | 0.00 | 6.39 ± 1.21* | 8.98 ± 1.05* | 10.53 ± 1.62* | 6.98 ± 0.72* |
| OO | 0.00 | 0.00 | 5.63 ± 0.92* | 10.62 ± 0.84* | 14.19 ± 1.15* | 8.21 ± 1.10* |
| HOSO | 27.18 ± 1.90 | 52.28 ± 2.88 | 8.83 ± 1.16* | 39.21 ± 2.35* | 54.35 ± 2.99* | 45.02 ± 2.70* |
| SO | 30.52 ± 2.14 | 44.81 ± 2.69 | 6.78 ± 0.98* | 31.31 ± 2.19* | 76.71 ± 3.45* | 85.01 ± 3.83* |
| Blend 5 | 0.00 | 0.00 | 0.77 ± 0.50 | 1.75 ± 0.23 | 2.24 ± 0.22 | 1.94 ± 0.26 |
| Blend 6 | 0.00 | 0.00 | 0.61 ± 0.42 | 2.74 ± 0.44 | 2.81 ± 0.66 | 1.96 ± 0.28 |
| Blend 7 | 1.18 ± 0.12 | 3.51 ± 0.67 | 0.93 ± 0.23 | 5.53 ± 0.21* | 6.32 ± 1.13* | 5.21 ± 1.08* |
| Blend 8 | 1.97 ± 0.29 | 2.38 ± 0.40 | 0.75 ± 0.40 | 2.95 ± 0.39 | 6.48 ± 0.91* | 8.08 ± 1.51* |
| Blend 9 | 0.00 | 0.00 | 0.45 ± 0.24 | 1.44 ± 0.22 | 1.88 ± 0.29 | 1.82 ± 0.28 |
| Blend 10 | 0.00 | 0.00 | 0.42 ± 0.22 | 2.10 ± 0.32 | 2.24 ± 0.35 | 1.76 ± 0.27 |
| Blend 11 | 0.57 ± 0.29 | 1.70 ± 0.26 | 0.49 ± 0.26 | 3.53 ± 0.55* | 3.95 ± 0.61 | 3.40 ± 0.53 |
| Blend 12 | 0.96 ± 0.22 | 1.17 ± 0.18 | 0.39 ± 0.20 | 1.95 ± 0.30 | 3.78 ± 0.58 | 4.77 ± 0.69* |
| Blend 18 | 0.11 ± 0.10 | 0.39 ± 0.21 | 0.10 ± 0.10 | 1.99 ± 0.31 | 2.17 ± 0.34 | 1.99 ± 0.31 |
| Blend 19 | 0.19 ± 0.10 | 0.22 ± 0.14 | 0.08 ± 0.10 | 1.18 ± 0.18 | 1.82 ± 0.28 | 2.19 ± 0.34 |

[†]mean, n = 8; *significant difference ($\alpha = 0.05$); VAO: Virgin Argan Oil; VOO: Virgin Olive Oil; OO: Olive Oil; HOSO: High Oleic Sunflower Oil; SO: Sunflower Oil; K: kaurene; UP: unidentified peak. See Table 1 for blend descriptions.

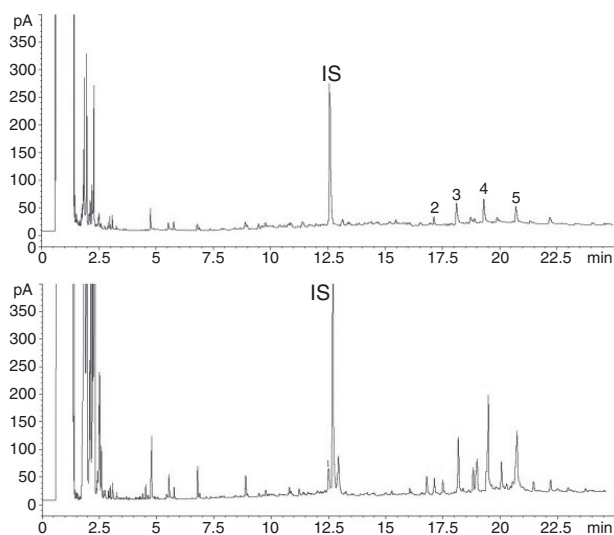


Figure 2
Example of GC chromatograms of the hydrocarbon fraction of VAO (a) and SO blend (b).

the means. In argan oil blends 11 and 7 at 10 and 5% of HOSO, C27 presented significant differences with the pure argan oil. Moreover, in 10% sunflower oil blends, significant differences were observed in the analysis of C29 and C31, and only the SO blend at 5% showed differences in C31.

3.4. Stigmastadiene compound

In order to detect the adulteration of virgin argan oil with refined oils (OO, HOSO and SO), we studied the stigmastadiene content, a steroidal hydrocarbon formed by dehydration of sterols especially from β -sitosterol. Figure 3 represents the gas chromatograms of virgin argan oil (Fig. 3a) and its blend with OO (Fig. 3 b). No appreciable content of

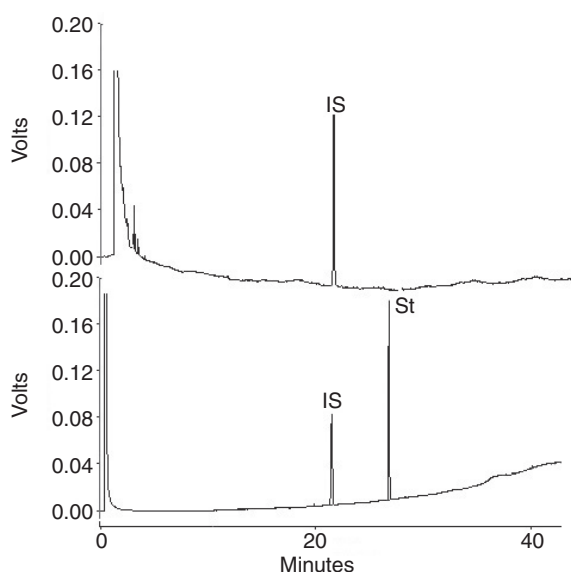


Figure 3
Example of GC chromatograms of the steroidal hydrocarbon fraction of VAO (a) and a refined OO (b). IS. cholesta-3,5-diene and 1. stigmastadiene.

stigmastadiene was observed for pure argan oil. Table 5 presents the results of the analysis of stigmastadiene in VAO, refined oils and their blends. The content of stigmastadiene at 5% HOSO and SO blends was 0.82 and 0.94 mg/kg respectively. Furthermore, in the case of olive oil containing 18.35 mg kg⁻¹ of stigmastadiene, the argan oil blends (5%) presented a stigmastadiene content of 0.88 mg/kg. All 5%-refined oil blends presented significant differences in the ANOVA test of stigmastadiene content. From the data reported in Table 5 it can be observed that simply by determining the stigmastadiene content in the studied argan oil blends it can be detected up to 5% of refined oils. We consider that Mass Spectrometry use in stigmastadiene determination could enable detection of the presence of refined edible oils at lower levels.

3.5. Alkyl esters of fatty acids

A bad conservation or over-maturation of olives increases the free fatty acid content and delivery of ethanol and methanol by fermentation. The content of alkyl esters (methyl and ethyl) is regulated for extra virgin olive oil and is limited by law. The aim of fatty acid alkyl ester determination in virgin argan oil was proposed to allow for the detection of mixtures with virgin olive oils of low quality. Figure 4 represents alkyl ester chromatograms of virgin argan oil (a) and a typical olive oil (b). Peaks corresponding to palmitic acid methyl and ethyl esters are present in VAO and OO. In addition, the

Table 5
3,5-stigmastadiene content in VAO and refined oils

| Sample [†] | 3,5-stigmastadiene (mg kg ⁻¹) | SD |
|---------------------|---|------|
| VAO | ≤ 0.1 | |
| OO | 18.95* | 0.76 |
| HOSO | 17.89* | 0.90 |
| SO | 20.12* | 0.79 |
| Blend 10 | 0.92* | 0.03 |
| Blend 13 | 0.63* | 0.03 |
| Blend 17 | 0.24 | 0.08 |
| Blend 11 | 0.88* | 0.06 |
| Blend 14 | 0.62* | 0.06 |
| Blend 18 | 0.24 | 0.08 |
| Blend 12 | 0.94* | 0.03 |
| Blend 15 | 0.61* | 0.03 |
| Blend 19 | 0.25 | 0.06 |

[†]mean, n = 8; *significant difference ($\alpha = 0,05$); VAO: Virgin Argan Oil; VOO: Virgin Olive Oil; OO: Olive Oil; HOSO: High Oleic Sunflower Oil; SO: Sunflower Oil. See Table 1 for blend descriptions.

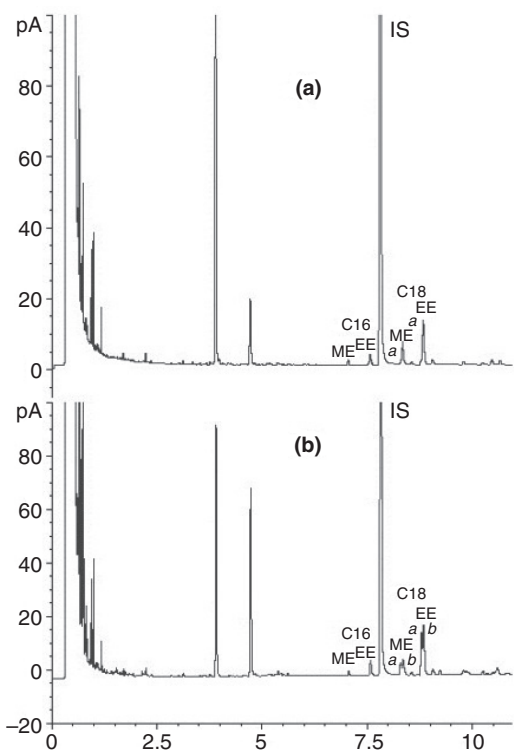


Figure 4
Alkyl ester chromatograms of VAO (a) and OO (b). Peaks correspond to C16:0 and C18 methyl and ethyl esters. $a = C18:1$, $b = C18:2$.

monounsaturated methyl and ethyl esters (oleic acid) are present in both oils (Figs. 4a and b), while linoleic acid methyl and ethyl esters are present only in virgin argan oil (Fig. 4b). The alkyl esters of the fatty acid profiles of both argan oil and olive oil are similar so this method is not able to detect argan oil adulteration with olive oil. We suppose that the presence of these compounds in the virgin argan oil could be due to inappropriate storage conditions of argan seeds. It would be interesting to study these compounds as possible quality parameter of virgin argan oil.

3.6. Chlorophyllic pigments

The color of virgin olive oil is the result of the combination of green and yellow colors due to the presence of chlorophyll and carotenoid pigments. In addition, chlorophyll is transformed by the action of temperature and acidic environment in pheophytin (pheo-*a*, *a'*, *b* and *b'*) which is quickly degraded by the action of different treatments in pirophoeofitine (Gandul-Rojas *et al.*, 2000). In order to detect possible argan oil blends with VOO, chlorophyllic pigments were determined. Figure 5 shows typical HPLC chromatograms of pigment extracts of the following oils: virgin argan, virgin olive and blend 1. Pheo-*a* was the main chlorophyll derivative present in virgin olive oil. The chlorophyllic pigments obtained by chromatographic separation of the pigment extracts of the studied oils are listed in Table 6. The occurrence of chlorophyll derivatives

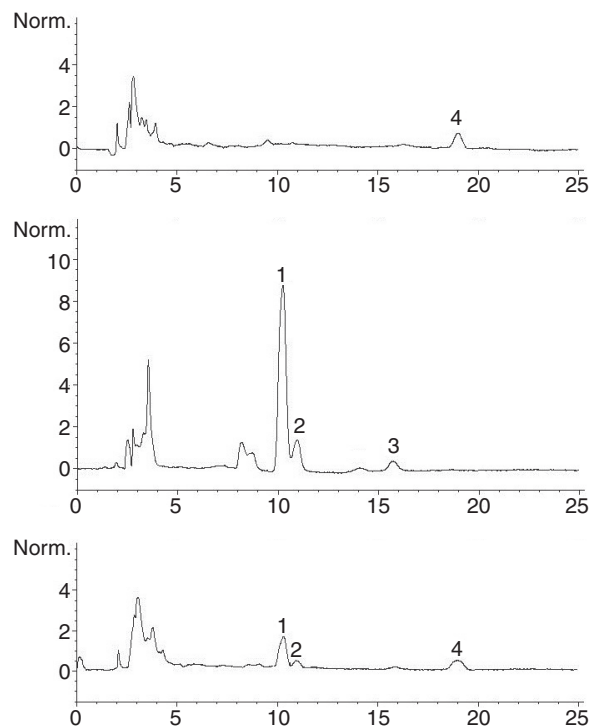


Figure 5
HPLC chromatogram (410 nm) of the chlorophyllic pigment extracts: VAO (a) VOO (b) and blend 1 (c). 1. pheophytin *a*; 2. pheophytin *a'*; 3. pyropheophytin *a* and 4. unidentified peak (RT= 18,97 min).

in VOO is in agreement with the previous findings. These compounds are absent in the virgin argan oil, due to their absence in its raw material, as well as other seed oils. The pheo-*a* analysis (Table 6) showed a 0.68 mg/kg content in virgin argan oil blends at 5% VOO. Therefore, pheophytin-*a* could be a possible marker of virgin olive oil contamination in virgin argan oil with up to 5%.

In agreement with the results found in this study it can be concluded that stigmastadiene, kaurene hydrocarbon and pheophytin-*a* can be used as possible markers for argan oil blends of up to 5%. Our findings show for the first time that stigmastadiene can be a potential marker to detect refined edible oil adulteration in virgin argan oil at a level of 3%, and an alternative to sunflower oil detection is the determination of the kaurene hydrocarbon. To detect virgin olive oils, pheophytin-*a* can be a possible marker. More highly sensitive analytical techniques are suggested for lower level adulterations of virgin argan oil (GC-MS, LC-MS).

The findings presented here may contribute to controlling the authenticity of pure argan oil, a valuable Moroccan product.

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Table 6
Chlorophyllic pigment contents in virgin oils and blends

| Sample [†] | pheophytin a (mg kg ⁻¹) | pheophytin a' (mg kg ⁻¹) | pyropheophytin a (mg kg ⁻¹) | RT= 18,97 ^{††} |
|---------------------|--|---|--|-------------------------|
| VAO | nd | nd | nd | 0,40 ^b |
| VOO | 12.55 ^a | 2.58 ^a | 0.80 ^b | nd |
| blend 9 | 0.68 ^b | 0.14 ^b | 0.05 ^b | 0.36 ^b |
| blend 16 | 0.14 ^b | 0.04 ^b | nd | 0.39 ^b |

[†]mean, n = 8; ^{††}unidentified peak; nd = not detected; aCV = 5%; bCV = 10%.;VAO: Virgin Argan Oil; VOO: Virgin Olive Oil. See Table 1 for blend descriptions.

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