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Review

# Authentication of vegetable oils by chromatographic techniques

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**Abstract**

Food authentication has been evolving continually to situations that were basically governed by a global market trend. Analytical techniques have been developed or modified to give plausible solutions to the devious adulterations at each moment. Classical tests have largely been replaced with newer technical procedures, most of which are based on gas chromatography, with some being based on high-performance liquid chromatography. Determination of *trans*-fatty acid and sterolic composition, together with sterol-dehydration products, have been used most frequently used to detect contamination and adulteration. Sophisticated new adulterations, e.g., olive oil with hazelnut oil, represent a new challenge for the next millennium, although suggestive proposals for detecting these kinds of adulterations are emerging with the contribution of databases and mathematical algorithms. 

*Keywords:* Reviews; Vegetable oils; Fatty acids; Triglycerides; Waxes; Sterols; Hydrocarbons; Alcohols; Vitamins

## Contents



## 1. Introduction

Oils and fats constitute one of the three major classes of food product, the others being proteins and carbohydrates. However, since the turn of the century, vegetable oils have supplanted lard and beef tallow as the major source of dietary fat. The change in consumer preferences is mostly due to consumers demanding food products that combine a pleasant flavor with nutritional benefits. On the basis of the expanding market for vegetable oils, their authentici-

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ty has become an important subject from both a commercial and a health perspective. The differential between the price of olive oil and other edible oils, for example, can mean that large amounts of money can be made by adding cheaper oils to virgin olive oil. This procedure, however, is also harmful since consumers buy olive oil for its health benefits [1] and are surprised to receive oil that does not provide them. The paradigm was the toxic oil syndrome resulting from consumption of olive oil spiked with aniline-denatured rapeseed oil that affected more than 20 000 people [2]. Authenticity [3] covers many aspects, including adulteration, mislabeling, characterization and misleading origin. With this plethora of possible issues and the great number of edible oils, it is not surprising that numerous methods have been used or suggested for the determination of oils during the past ten years. Monitoring the authenticity of edible oils is carried out using instrumental techniques that provide data about their qualitative and quantitative composition. The advances in knowledge and technology have undoubtedly led to greater success in the fight against adulteration over the years. However, it is equally true that the same techniques and knowledge have also been used by defrauders in order to invalidate the usefulness of some methods. Adulteration with desterolized oils are not detected by the procedure based on the sterolic fraction determination. Such competition requires not only a considerable investment in perfecting or developing new techniques, but also a requirement to keep up with research and development is that malpractices can be detected. As a result of the advances in analytical methods, or the new challenges created by fraudsters, official methods and trade standards are periodically revised and upgraded.

Most of the current work on edible oil adulteration is based on chromatographic analysis. High performance liquid chromatography (HPLC) and high-resolution gas chromatography (HRGC) analyses have been applied to the quantification of fatty acids, triglycerols, sterols, tocopherols and hydrocarbons [4]. Although HPLC has been used increasingly over the last decade, HRGC is still the most widely used technique. However, these separative techniques have been complemented with, or substituted by, many other modern techniques, such as supercritical fluid chromatography (SFC), chiral chromatography, silver ion chromatography, stable carbon isotope ratio analysis (SCIRA), mass spectrometry (MS), nuclear magnetic resonance spectrometry (NMR), near-infrared spectroscopy (NIR), Fourier transform infrared spectroscopy (FT-IR) and FT-Raman spectroscopy [5–13].

The purpose of this paper is to present the current contribution, and recent trends, in chromatography to the authentication of vegetable oils by reviewing the most recent papers on the quantification of chemical compounds present in edible oils (Table 1). Because of the numerous components of vegetable oils, many analytical procedures have been employed to identify and quantify them. For organizational reasons, this paper has been structured based on the chemical compounds. Following traditional divisions, there are two groups. The saponifiable matter made up of triglycerides, partial glycerides, esters of fatty acids with saturated fatty alcohols of linear chain, terpenic alcohols and free non-esterified fatty acids. The unsaponifiable matter, which makes up around 2% of all oils, includes many chemical substances of very different structure, such as alcohols of triterpenic structure, hydrocarbons, 4,4-methyl-sterols, phenols and flavonoids, sterols, pigments, terpenic dialcohols, tocopherols and volatile compounds. For those series that are not analyzed, there are recent interesting reviews [14,15].

## 2. Fatty acids

The edible oil fatty acids have always been one of the main issues because of their importance in the description and determination of adulteration. Fatty acids, the main components of any edible oil, are usually converted to fatty acid methyl esters (FAMEs) for gas–liquid chromatography (GLC) analysis using capillary columns. Methyl esters are prepared by methylation in a methanolic medium that can be carried out with alkaline, acid, or alkaline and acid catalysis. Other alternatives are methylation with diazomethane, which is used for free acids [16], and the formation of methyl esters without previous extraction of the fat [17], where both methylation with methanol and acid catalysis are carried out in a dissolving phase. The use of capillary columns in

Table 1
Overview of methods employed to determine the authenticity of vegetable oils[a]

| Issue | Apparatus | Applicability | Analyte-indicative information | Reference |
|---|---|---|---|---|
| A | Various | Any kind of edible oil in olive oil | Fatty acids, LLL, ECN-42 sterols, hydrocarbons, etc. | [28] |
| A | LC–GC | Desterolized sunflower, soybean, palm, grapeseed oils in ROO | 3,5-Stimastadiene/3,5-campestadiene | [92] |
| A | LC–GC | Reesterified edible oils in olive oil | Fatty acids in 2-position of triglycerides | [30] |
| A | LC–GC | Refined rapeseed, soybean, sunflower, grapeseed, palm oils in VOO | Sterols, ratio oleic/linoleic | [21,83] |
| A | LC–GC | Desterolised sunflower oil in olive oil | $\Delta^8$(14)-Stigmastenol | [94,96] |
| A | GLC | Palm olein in cottonseed oils | Palmitic acid, $C_{50}$ and $C_{54}$ carbon number triglyceride | [60,61] |
| A | GLC | Palm kernel olein in palm kernel | Oleic acid | [149] |
| A | GLC | ROO in VOO | Fatty acids | [140] |
| A | GLC | Sunflower or corn oils in olive oil | Sterols | [142] |
| A | GLC(+NMR) | Hazelnut in olive oil | Fatty acid profile | [25] |
| A | GLS | Borage oils in evening primrose oil | Erucid and linoleic acid | [23,148] |
| A | HRGC | ROO or ROPO in VCO | Stigmastadienes | [86,89] |
| A | HRGC | HOSO in VOO | Stigmastadienes | [28,86,89] |
| A | LC-GC-MS, HPLC | Refined oils in VOO | Stigmastadienes | [90,91] |
| A | HTC–GC | Refined soybean oil in ROO | Atom carbon number | [52] |
| A | GC–EIMS | Rapeseed oil in ROO | Brassicasterol | [82] |
| A | SDE-GC, SFE-GC, RPLC-GC | Hazelnut oil in olive oil | Fibertone | [111,112,152] |
| A | GC–C-MS | Any edible oil in corn oils | $^{13}C/^{12}C$ ratios | [36] |
| A | | Blackcurrant in evening primrose oil | Stearidonic acid, tocopherols | [23,24,148] |
| A | HPLC, HRGC-MS | Hazelnut in olive oil | Ratio $\gamma/\beta$ tocopherols | [105,150,151] |
| A | HPLC+GC | Linseed oils in soybean oils | Gramisterol and tocopherols | [104] |
| C | HPLC | Soybean or sunflower oils in olive oil | Triacylglcerols | [128] |
| | | Non-natural products in VOO | Chlorophylls | [137] |
| C | HPLC | Refined corn, cottonseed, sunflower, soybean and rape-seed oils in ROO | Triacylglycerols | [141] |
| C | RP-HPLC | Canola oil in VOO or ROO | Triacylglcerols | [57] |
| C | RP-HPLC | Palm or grapeseed oils in olive oil, soybean etc. | Tocopherols, tocotrienols | [100] |
| | Various | All olive and olive–pomace oil categories | Fatty acids, LLL, ECN-42 sterols, alcohols, hydrocarbons, etc. | [28,129,130,134] |
| C | GLC | VOO of Spanish olive varieties | Fatty acids, sterols, alcohols, triglycerides, hydrocarbons, etc. | [97,107,133–135] |
| C | DHS–GC | Spanish, ltalian, Greek varieties | Volatiles | [97,106,107,121,122,139,143] |
| C | HRGC | Selected European varieties | Fatty acids, sterols, volatiles, alcohols, etc. | [97,107,124] |
| C | HRGC | Extraction system: pressing, percolation, centrifugation. | Aliphatic alcohols | [119,136] |
| C | HRGC | Geographical Origin: Denominations of Origin, counties, regions, and countries. | Different components according to the authors | [85,114,116–118,120,123, 125–127,132,138, 144–147] |
| C | GC–C–SIR–MS | Groundnut, palm, rapeseed, sunflower oils | Ratio $^{13}C/^{12}C$ | [35] |
| C | HPLC+GC | vegetable oils | Steryl esters | [84] |
| C | HPLC | Categories of VOO and ROO | Triglycerides | [131] |

[a] A, authentication; C, characterization.

DHS, dynamic head space; ECN, equivalent carbon number; GC-C-SIR-MS, gas chromatography combustion stable isotope ratio mass spectrometry; GC-EIMS, gas chromatography electron ionization mass spectrometry; HOSO, high oleic sunflower oil; LLL, trilinolein; NMR, Nuclear Magnetic Resonance; ROO, refined olive oil; ROPO, refined olive-pomace oil; RPLC, reversed phase liquid chromatography; SDE, simultaneous distillation-extraction; SFE, supercritical fluid extraction; VOO, virgin olive oil.

GLC analysis has been widely accepted. The use of open capillary columns has notably improved the resolution of separations, improving the precision of analysis in terms of quality, quantity, sensitivity, and analysis times, among others. American Oil Chemist's Society (AOCS) official methods include various procedures for FAME quantification by GLC with capillary columns [18]. Reviews have been published on various aspects of the preparation [19,20].

The composition of fatty acids has traditionally been used in the food industry as an indicator of purity, although the wide variation in edible oils from different geographical origins is a limiting factor in the interpretation of data with regard to adulteration. Some kinds of contamination can be detected, however, or, at least, can give clues for additional tests. Palmitic acid is used as an indicator of adulteration of cottonseed oil by palm olein, since cottonseed oil has a palmitic acid content of between 21 and 26% whereas palm olein contains around 40% palmitic acid. Linoleic acid content is a good indicator of purity if groundnut and sunflower oils are suspected of being adulterated with cheaper soybean or rapeseed oils. Sunflower and groundnut oils contain less than 0.1% of this acid vs. 10% in rapeseed and soybean oils. The linoleic acid content and the ratio of oleic to linoleic were used to detect mixtures of virgin olive oil with 5–10% refined seed oils [21]. Information regarding oleic and linoleic acids allows for the detection of soybean adulteration with rapeseed oil. Rapeseed oil contains about 50% oleic acid whereas soybean oil contains 20%. Conversely, the linoleic acid content in soybean oil is around 40% whereas in rapeseed oil, it is about 20%. The addition of palm kernel olein to palm kernel oil can be detected by determining the fatty acid composition, as the olein contains higher amounts of oleic acid and has a higher iodine value [22]. This procedure can be used even after hydrogenation, if the total amount of $C_{18}$ acids is calculated and compared with standard ranges. Erucic acid is used to detect if evening primrose oil has been fortified with borage oil since the first has no erucic acid [23] and the second contains between 3 and 5%. The addition of blackcurrant seed oil to evening primrose oil can be detected by determining stearidonic (C18:4) acid, as the first contains 3–4% [24] whilst the second does not have this acid. Mannina and coworkers [25] have used a fatty acid profile (palmitic, palmitoleic, eicosanoic, eicosenoic and linolenic acids), together with the statistical procedure of linear discriminant analysis, to detect adulterations of olive oil with hazelnut oil. Around 10% adulteration could be detected, although more work is needed with other samples due to the wide ranges of fatty acid composition in olive oils [26].

Geometric isomers and double-bond position isomers can be resolved using very long capillary columns. Until recently, the determination of *trans*-fatty acids was confined to the use of infrared spectroscopy [27]. At present, gas chromatography allows not only the percentage of *trans*-fatty acids to be determined but they can also be identified and the proportion of each one of them to be calculated. Cold methylation, with methanolic potassium hydroxide, and making sure that the injector is kept clean (so avoiding an increase in the amount of elaidic acid) are recommended in order to obtain a good correlation with infrared measurements. The amount of sample injected should be such that the arachidic acid peak is between 20 and 50% of the full scale. The appearance of peaks (ethyl or other esters) that interfere with *trans*-linolenic acid can be due to the use of columns with less polarity. The injection of a previously contrasted sample (refined vegetable sample deodorized at 260°C for 5–8 h, or an edible oil mixed with hydrogenated soybean oil) would allow the determination of the position of *trans*-isomers.

The presence of the *trans*-isomers of oleic, linoleic and linolenic acids in olive and olive–pomace oils, above the maximum levels [28], can indicate adulteration with hydrogenated seed oils [29], esterified olive oils [30], illegally treated virgin olive oils [31] or mutant (or genetically altered) seed oils desterolised at high temperatures [32]. The presence of deodorized oils can be identified by the positional double bond isomers of linoleic acid, quantified from methyl esters by HRGC, although the admixture of virgin olive oil with oils deodorized with alkali-washing can be detected by the determination of diglycerides. If the amount of diglycerides is not equivalent to the oil acidity, then free fatty acids have been removed during a refining process, indicating the presence of refined oil [33].

There has been some interesting and promising new research devoted to the characterization of oils, mostly olive oil, used in canned fish. Colonello et al. [34], for instance, used a TLC $AgNO_3$ technique together with GLC (column, Supelco SP-2340) to conclude that the presence of $C_{18:1}$ *trans*-isomers in the composition of fatty acid oils should be carefully considered when judging the authenticity of the covering oil. Coupled techniques have also been used to detect adulteration. Gas chromatography–combustion–stable isotope ratio mass spectrometry (GC–CSIR–MS) has been used to investigate the authenticity of groundnut, palm, rapeseed and sunflower oils [35]. Oils were derivatized and their carbon isotope ratios ($^{13}C/^{12}C$) were calculated. Canonical discriminant analysis allowed the separation of sunflower oils from other varieties of oil. A large database containing 150 vegetable oils characterized by their fatty acid composition $\delta^{13}C$ values of the major fatty acids ($C_{16:0}$, $C_{18:1}$, $C_{18:2}$) were used to authenticate commercial maize oil [36]. Gas isotope ratio mass spectrometry was employed and the $\delta^{13}C$ values of fatty acids were related to a combination of genetic (variety) and environmental (geographical origin and harvest) factors.

## 3. Triglycerides

Triglyceride composition has also been established as a measurement of the quality and purity of vegetable oils. Because of the specificity of the composition in different kinds of fats and oils, it is used increasingly in the food industry to confirm authenticity despite this type of analysis being quite laborious. Analysis can be carried out by HPLC or high-temperature (about 350°C) GLC, although HPLC is becoming widely accepted in the analysis of triglycerides [37–42]. Different types of sample preparation, stationary and mobile phases, and detectors have been studied to separate triglycerides according to the number of carbon atoms by HPLC. The greatest resolution of a triglyceride homologous series, regardless of the vegetable oil, was found using an RP-18 (Spheri 5 μm) column. Reversed-phase columns, with the addition of silver ion, has been suggested by Plattner [43], and Neff et al. [44] used a silver ion HPLC system with flame ionization detection in the characterization of various edible oils. Depending on the degree of separation demanded and the type of detection used, any one of the following four mobile phases can be used used (i) acetonitrile–acetone, (ii) acetonitrile–isopropanol, (iii) acetonitrile–dichloromethane, and (iv) acetontrile–ethanol–hexane. The detectors commonly used are ultraviolet (UV) and refraction index (RI), the first being the most sensitive. However, the UV detector presents problems with the isomerization and conjugation of double bonds, and it is not the most widely used today. An alternative is light-scattering detection [45], although some problems have also been found using this method [46]. The RI detector is the most appropriate for quantitative analysis if the analysis is carried out using thermostated cells and isocratic elution, as the detector is greatly affected by the temperature and the composition of the mobile phase. The operating conditions for reversed-phase HPLC depend on the application. For example, a 1.15-ml/min flow-rate of acetonitrile–acetone (50:50, v/v) has been suggested by Morales and León-Camacho [46] to get the best resolution of the equivalent carbon number (ECN) peaks ECN-42, while acetonitrile–dichloromethane (75:25, v/v), at a flow-rate of 0.9 ml/min, was suggested by Graciani-Constante and Delgado Noriega [47] to improve the resolution of ECN-44 and ECN-46. El-Hamdy and Perkins [48] used the ECN to cluster triglycerides exhibiting the same behavior on reversed-phase HPLC columns. The ECN is the actual carbon number minus twice the number of double bonds per molecule. The application of ECN to the detection of admixtures of vegetable oils has been successful. Kapoulas and Andrikopoulos [49], for example, reported that the presence of 2.5% sunflower oil and 3–4% of other linoleate-rich oils in olive oil can be unequivocally detected using their ECNs (ECN 42, 44 and 48).

Concerning gas chromatography, IUPAC has adopted a method to resolve triglycerides according to the number of equal carbon atoms by GLC [50]. High-resolution GLC offers interesting possibilities (efficiency, expediency and stability), although there are some problems associated with the injection system, deterioration of the column's stability with temperature [46], and alteration of compounds during analysis [51]. However, the technique is able to

resolve triglycerides both by atom carbon number and by unsaturation [52,53] using phenyl-methyl-silicone stationary phases [54].

From the point of view of authentication, fatty acids are distributed on glycerol molecules according to certain position-specific patterns and, hence, triglycerides are considered to be good fingerprints for adulteration purposes. The 'even' and 'restricted random' distribution theories suggest that the qualitative profile of triglycerides is not affected by natural quantitative variations in their contents of individual fatty acids [55]. Thus, the acids at the 2-position come from a different biochemical pool from those at the 1- and 3-positions, and have a different composition. If a sample of sunflower or groundnut oil, which should contain traces or small quantities of $C_{18:3}$, is found to contain this acid, the determination of the ratio of $C_{18:3}$ at the 2-position to the overall concentration of this acid will help to determine if it was adulterated with either rapeseed or soybean oil. The low level of trilinolein (LLL) in olive oil has been used to confirm its authenticity and to detect adulteration [56], although Salivaras and McCurdy [57] have reported that admixtures of olive oils with less than 7.5% canola oil could not be detected. Their finding is in agreement with that of Proto [58] who reported that the low content of LLL in some seed oils would allow adulterations that were undetectable by LLL quantification. Triglyceride analysis is however useful for identifying the presence of re-esterified oils in olive oil. Gegiou and Georgouli [30] pointed out the differences between olive oils and re-esterified oils with regard to the following ratios, 1-oleo-2,3-dipalmitin to 1,3-dipalmito-2-olein (8:92 for olive oil and 55:45 for re-esterified oils), 1,3-dioleo-2-palmitin to 1-palmito-2,3-diolein (7:93 vs. 55:45), and 1,3-dioleo-2-stearin to 1-stearo-2,3-diolein (16:84 vs. 46:54). El-Hamdy and El-Fizga [59] detected adulterations of olive oil with about 1% of linoleic-rich vegetable oils (soybean, sunflower and corn oils) although detection of olive oil admixed with non-rich linoleic acid seed oils is more difficult.

The success found with ECNs [48] lead to its application in trade standards and official methods. The difference between the empirical and theoretical ECN 42 triglyceride content is a European Union (EU) official method since 1997 [28]. The official method is an invaluable tool for detecting the fraudulent addition of seed oils to olive oil even at low percentages. On the subject of seed oils, the adulteration of the more expensive safflower oil with sunflower oil can be detected by determining the carbon number triglyceride composition, since the $C_{60}/C_{58}$ ratio for safflower oil is always over 1.0, often over 2.0, whilst it is always below 1.0 for sunflower oil. The adulteration of cottonseed oil by palm olein can be clarified by determining the ECN, since cottonseed oil contains 12.6–19.9% $C_{50}$ (palm olein, 37.7%) and 32.2–43.6% $C_{54}$ (palm olein, 12.8%) [60,61]. Palm oil contaminated with stearins or oleins can be detected by the product of palmitic acid enrichment factor and the $C_{48}$ (tripalmitin) carbon number concentration.

## 4. Waxes

The content of waxes varies among the various categories of olive oil [28]. Hence, the profiles of these esters of fatty acids and fatty alcohols are of interest as indicators of both quality and purity. Virgin olive oil can be distinguished from refined olive oil and olive–pomace oils because the first has a higher content of $C_{36}$ and $C_{38}$ waxes than of $C_{40}$, $C_{42}$, $C_{44}$ and $C_{46}$ whilst the other oils have an inverse relation. The most common methodologies are based on separation by liquid chromatography followed by gas chromatographic analysis. Mariani et al. [62] optimized a GLC methodology for quantifying waxes together with other series of chemical compounds, such as sterols, alcohols, tocopherols and squalene. Alternatives such as thin-layer chromatography [63], open column chromatography [64] and off-line HPLC methods [65] have all been used for the isolation of the total wax ester fraction. GC analysis is then carried out to quantify each compound. Imperato [66] studied the reproducibility of the official methodology [67] while León-Camacho and Cert [68] analyzed different solutions for the usual drawbacks.

Detailed studies on the chemical structure of long chain esters from pomace oil have also been made using GC–MS [69]. The oleic acid esters, with short ($C_1$–$C_6$) and long chain ($C_{22}$–$C_{28}$) aliphatic alcohols, benzyl alcohol esters, with $C_{26}$ and $C_{28}$

saturated fatty acids, and steryl esters [70] are ester classes that have been detected in the waxy fraction of the whole olive fruit [71]. In this respect, the determination of waxes in olive oil and olive–pomace oil (≤250 ppm for extra-virgin, virgin and ordinary virgin olive oils, and ≤350 ppm for other categories) have been regulated by an EU directive [28] with the objective of detecting the possible presence of olive–pomace oils. Difficulties can arise however with those virgin olive oils with waxes close to the maximum content (<250 ppm) since esterified compounds (such as waxes and sterols esters) increase during storage by up to twice the initial amount in 18 months [72]. Thus, a positive result of this methodology should be checked by the quantification of terpenic dialcohols.

## 5. Sterols

Sterols, which comprise a major portion of the unsaponifiable matter, are found in almost all fats and oils, and they are also characteristic of the genuineness of vegetable oils. Rapeseed oils, for example, contain significant levels of brassicasterol (100–1100 mg/kg for canola), while olive oil has high levels of β-sitosterol (683–2610 mg/kg) and $\Delta^5$-avenasterol (34–266 mg/kg), and safflower and sunflower seed oils have high levels of $\Delta^7$-stigmastenol (300–550 and 150–500 mg/kg, respectively). These apparent differences make them the most suitable for determining the botanical origin of oils and, hence, for detecting adulteration/contamination among vegetable oils. Chromatographic methods are currently the most widely used for the qualitative and quantitative analysis of this extensive series of compounds clustered in 4-demethylsterols or simply sterols, 4-monomethylsterols, and 4,4-dimethylsterols or triterpene alcohols. Gas chromatography of unsaponifiable matter is the prevalent technique, although other methodologies have been suggested [73–78]. The unsaponifiable matter is isolated preferably using the diethyl ether procedure that allows the total extraction of sterols [46]. Various developing liquids can be used to purify this material by thin-layer chromatography on silica gel. The free fatty acids are retained in the unsaponifiable fraction by impregnating the plate with alcoholic KOH. Injection is usually performed after derivatization with a silanizing reactant. Capillary columns give the best performance since they can resolve the sterols almost completely [79], which is almost impossible with packed columns. Morales and León-Camacho [46] have discussed practical solutions to the current problems of analyzing the sterols of olive oil. while Gerst et al. [80] studied the utility and limitation of this technique in their GC and GC–MS analysis of unsaturated $C_{27}$ sterols.

As stated above, the profile of sterols is almost decisive in clarifying the contamination of some vegetable oils with other cheaper ones. Thus, the addition of more than 5% of rapeseed oil to sunflower or groundnut oils would be detected by the brassicasterol since rapeseed oils contain over 100 mg/kg and the spiked oils less than 0.2 mg/kg. Brassicasterol is also useful for the determination of the adulteration of rapeseed oil with soybean oil. Adulteration of safflower oil by sunflower oil can be detected by determining the concentration of monomethyl sterols, since sunflower oil has much higher levels. Suspected additions of borage oil to evening primrose oil can be detected by determining $\Delta^5$-avenasterol (15–28% of total sterols for borage oil vs. around 4% for evening primrose oil), plus 24-methylene cholesterol. The high content of $\Delta^7$-stigmastenol in sunflower and safflower oils (16–23% for safflower oil, 13–18% for high oleic safflower oil, and 7–13% for sunflower, and 14–22% for high oleic sunflower oil) can help detect their addition to other vegetable oils (e.g. corn, olive oil, canola). Adulterations of corn (maize) oil could be detected by determining the sterol profile together with that of tocopherols and fatty acids, although they overlap with those of other vegetable oils. The best results however are obtained by stable carbon isotope ratio analysis (SCIRA), which allows the identification of as little as 7% impurity [81].

Brumley et al. [82] detected the presence of brassicasterol in olive oil by GC–electron ionization mass spectrometry (GC–EI–MS). Grob et al. [83] were able to detect adulteration of virgin olive oil with seed oils (rapeseed, soybean, sunflower and grapeseed) by direct analysis of the sterols using on-line coupled LC–GC–flame ionization detection (FID). Additions of around 2% rapeseed oil were detected by determining the content of brassicasterol

(≤0.1% of total sterols for olive oil vs. 12–13% for rapeseed oil and 5–13% for canola oil). The addition of 10% soybean oil was detected by determining the concentration of campesterol (15–24% for soybean oil vs. ≤4% for olive oil) and stigmasterol (16–19% for soybean oil vs. less than campesterol for olive oil). Contamination of sunflower oil higher than 5% could be detected by the increase of campesterol (7–13% for sunflower oil and 8–10% for high oleic sunflower oil), stigmasterol (8–11% for sunflower oil), and $\Delta^7$-stigmastenol (7–13% for sunflower oil and 14–22% for high oleic sunflower oil vs. ≤0.5% for olive oil) concentrations. Adulteration with 10% or higher of grapeseed oil would increase the concentrations of campesterol (9–14% for grapeseed oil) and stigmasterol (9–17% for grapeseed oil) but an admixture of around 5% would be at the limits of detection with this methodology. An alternative to these kinds of sterols is offered by steryl esters, which are complex mixtures that differ in composition from that expected by a random esterification of total fatty acids and sterols in the oil. Gordon and Miller [84] have used HPLC and GC to quantify these compounds. Principal component analysis (PCA) was able to establish clear differences among seed oils, e.g. between corn, rapeseed and sunflower oils. They defined three groups, oils (corn and rapeseed oils) with a high content (<4000 mg/kg) of steryl esters, oils (sunflower and high-oleic sunflower oils) with a medium content (1400–2400 mg/kg), and oils (safflower, soybean, cottonseed, groundnut, olive and palm oils) with a low content (<1200 mg/kg) of steryl esters.

## 6. Hydrocarbons

Small amounts of normal, saturated, terpenic and even aromatic hydrocarbons are always present in virgin olive oil [26,85]. During the refining processes (decoloration and deodoration), however, sterols undergo dehydration reactions that result in the corresponding unsaturated hydrocarbons possessing the steroidal nucleus. Lanzón et al. [86] described how each sterol gave rise to their corresponding dehydrated hydrocarbon. The sterol β-sitosterol produces 3,5-stigmastadiene, campesterol produces 3,5-campestadiene, and stigmasterol produces 3,5,22-stigmastatriene. The trace yielded by sterols during the bleaching step [87,88] would allow the detection of admixtures with vegetable oils where these minor compounds have been removed by a strong refining process. Lanzón et al. [89] were pioneers when they suggested the use of stigmasta-3,5-diene as a marker for detecting admixtures of virgin olive oil with refined vegetable oils. The technique employed was gas chromatography of the hydrocarbon fraction isolated from the unsaponifiable matter. The oil was saponified and extracted with hexane. The extract was cleaned up on a silica gel column by eluting it with 1 ml/min hexane, and fractions (25 ml) were collected and concentrated to 1 ml. Each fraction was separately analyzed by gas chromatography. Today, the steroidal hydrocarbons can also be determined by on-line LC–LC–GLC–MS [90], and by HPLC on an RP-18 column and using UV detection at 235 nm [91].

The ratio of the degradation products of sitosterol and campesterol is a good marker for desterolized sunflower, soybean, palm or grapeseed oil [92], while the determination of 3,5-cholestadiene allows the evaluation of lards deodorized or bleached by activated earths [93]. The sterol dehydration products have been also used to detect the presence of desterolized rapeseed oils by quantifying campestatriene. Mariani et al. [94] set up a technique for the separation of $\Delta^{8(14)}$- and $\Delta^{14}$-sterols (produced through isomerization [95] from $\Delta^5$-sterols) making it possible to determine small additions of desterolised high oleic sunflower oil to olive oil. $\Delta^7$-Stigmastenol and its derivative $\Delta^{8(14)}$-stigmastenol have also been detected by coupled methods using LC–GC–FID [96].

## 7. Alcohols

The determination of aliphatic and triterpene alcohols and the triterpene dialcohols erythrodiol and uvaol are also used for ascertaining the genuineness of olive oil [97]. Studies using combined column–thin-layer chromatography have been conducted on the aliphatic linear and triterpenic alcohols from virgin olive oil, determining their composition by GC analysis using both packed and capillary col-

umns [64,98]. The TLC separation of linear and triterpenic alcohols from each other is difficult although procedures have been suggested [46]. The analytical determination of pentacyclic triterpenic alcohols is carried out using the sterolic fraction and capillary columns, and they can be quantified using betulin as an internal standard.

The content of triterpene dialcohols in extra virgin olive oil must not exceed 4.5% of total sterols as higher values would indicate blending with olive-pomace oil [28]. Positive results should be checked with the wax content information available. However, this percentage has been discussed [99] as some genuine Spanish virgin olive oils would be considered adulterated.

## 8. Tocopherols

These natural antioxidant compounds, which give stability to oils, vary from one edible vegetable oil to another. Sunflower oil contains less than 50 mg/kg of γ-tocopherol and soybean oil contains up to 2400 mg/kg, while the total content of tocopherols (α-, β-, γ- and δ-tocopherol) olive oil varies between 50 and 150 mg/kg. Tocopherols can be analyzed, after the saponification reaction, by reversed-phase HPLC with amperometric detection [100], and by the direct injection of the diluted sample into a reversed-phase HPLC system coupled to a UV detector [101]. Besides the HPLC standard method, which is accepted by IUPAC [102], a gas chromatographic procedure is also used [103]. The concentration of δ-tocopherol is used to detect the suspected contamination of groundnut oil (<22 mg/kg) with soybean oil (932 mg/kg). The addition of palm oil and grapeseed oils to any tocotrienol-free vegetable oil (e.g. olive oil) can be detected at 1–2% [100]. Manandhar et al. [104] used tocopherols to detect the adulteration of soybean oils with linseed oils, although the best compound was the sterol gramisterol. Recently, the content of tocopherols has been investigated [105] to detect the sophisticated adulteration of olive oils with hazelnut oils. Olive oils contain a higher percentage of β-tocopherol than γ-tocopherol compared with hazelnut oils. Conversely, the olive oils have traces of δ-tocopherol whereas hazelnut oils contain higher quantities. The authors have suggested that genuine olive oils should have a value lower than five for the ratio between γ and β-tocopherols.

## 9. Volatiles

The control of compounds responsible for the flavor is currently routinely performed by the food industries. In this context, several approaches have been developed in order to characterize [106] or authenticate oils [107]. Most of these procedures are based on enantioselective analysis and are applied to essential oils using GC–isotope ratio MS with a combination of a non-chiral precolumn and a chiral main column [108] or enantioselective multidimensional gas chromatography [109]. The practical possibilities of using volatile compounds in the authentication of virgin olive oil varieties would open up a new way of detecting the adulteration of virgin olive oil with hazelnut oil. Filbertone, (*E*)-5-methylhept-2-en-4-one, had been identified as the most important volatile compound in hazelnut oil and has a great flavor-impact in either crude or roasted hazelnuts [110]. Blanch et al. [111] have tested different techniques to determine their suitability for the detection of filbertone. RPLC–GC was the most satisfactory for detecting compositional differences in olive oil and hazelnut oil. New approaches have just been developed using distillation followed by gas chromatography with a chiral-column [112], or a treatment of the sample previously to the volatile concentration by purge-and-trap, and the volatile quantification by gas chromatography with a DBWax column [113].

## 10. Conclusions

The interest of researchers in the authentication of vegetable oils has led to an improvement in the control of adulteration. Most of the resulting issues and methodologies are described in the official methods and trade standards published by the Association of Official Analytical Chemistry, American Oils Chemists' Society, International Olive Oil Council, International Union of Pure and Applied Chemistry, Federation of Oil Seeds and Fats As-

sociations, and/or the Commission of the European Communities, among other institutions. Official methods are based on maximum or minimum limits of certain chemical components and, unfortunately, this means that the adulteration can be carried out undetected so long as the limits prescribed are not exceeded. Many fraudsters have information on the chemical composition of edible oils (either raw or refined) produced throughout the world and, using a computerized program, they could prepare fraudulent recipes that would be undetected using the current official methods. Paganuzzi [32] describes a nice example of a hypothetical sophisticated adulteration (65% refined olive oil, 15% refined hazelnut, 15% desterolized sunflower and 5% palmolein without free sterols) that would be undetected under the current legislation inside the EU [28]. Powerful mathematical tools should be used to control the current sophisticated adulterations. The analytical procedures, either official or in-house, should be completed with large databases, with the chemical composition of edible oils and experts systems [114–116]. The intelligent decision systems could suggest the most adequate analysis at each step of an authentication study and build the final conclusion about the existence or absence of adulteration. On the other hand, the rapid development of emergent techniques suggests the coupling of spectroscopy with chromatography. Chromatographic techniques could be used to quantify those chemical compounds that are markers for adulteration, and their results could be applied to the multivariate calibration of the spectroscopic techniques.

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