# We are IntechOpen, the world's leading publisher of Open Access books
# Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

154
Countries delivered to

Our authors are among the
TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 6

# Pigments in Extra-Virgin Olive Oil: Authenticity and Quality

Cristina Lazzerini, Mario Cifelli and
Valentina Domenici

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64736

**Abstract**

Pigments, divided into carotenoids and chlorophyll derivatives, are responsible for the colour of extra-virgin olive oil (EVOO). The concentration of pigments in EVOO depends on several factors, such as the maturity of olives before oil production, the cultivar and the geographic origin of olives. Pigments naturally degrade in olive oil (OO) during storage, and they may decompose due to light, temperature and oxygen exposure. The nature and concentration of pigments in EVOOs are different from seed oils, and this is a base of their use to reveal oil treatments and sophistication. In this chapter, the analytical methods, mainly chromatographic and spectroscopic, applied to identify and quantify pigments are overviewed. In particular, the applications of these methods to check the authenticity and the quality of extra-virgin olive oil are discussed.

**Keywords:** carotenoids, chlorophylls, quantitative methods, adulterations, EVOOs

## 1. Introduction

The authenticity of edible olive oils is a subject of intense research all over the world. The aim is to guarantee the protection of consumers and to avoid frauds damaging not only the consumers but also the producers. Olive oil (OO), and in particular extra-virgin olive oil (EVOO), is one of the most susceptible to fraud foods. There are many different frauds which can be roughly divided into [1]:

- *Adulteration*: at least a component is substituted with one of a lower value;

INTECH
open science | open minds

© 2016 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

- *Tampering*: legitimate products are used in a fraudulent way;
- *Overrun*: a legitimate product is produced with a certain amount in excess with respect to product agreement;
- *Diversion*: the sale or distribution of legitimate products out of the intended market;
- *Simulation*: illegitimate products are designed to look like but not exactly copy the original product;
- *Counterfeit*: all aspects of the fraudulent product are fully replicated.

All the above frauds are usually intentional, and the main motivation is economical.

Most of the known frauds in the field of EVOOs concern their saponifiable fraction, which represent the 98–99% of olive oil. It is composed of saturated and unsaturated fatty acids, esterified almost entirely to glycerol to form triacylglycerols. Diacylglycerols, monoacylglycerols and free fatty acids are also components of the saponifiable fraction. The unsaponifiable fraction is composed of a very large number of minor compounds, very important for the flavour and the nutritional properties of EVOOs [2]. Minor components of olive oil, which include phenols, aliphatic and other alcohols, hydrocarbons, tocopherols, sterols and those responsible of the colour [3], namely the pigments, can be the object of alterations and frauds, too. An example is the addition of an artificial pigment, called E141, which is similar to chlorophyll, where the inner metal ion, $Mg^{++}$, is substituted with the more stable $Cu^{++}$. This fraud can be unmasked by a simple spectroscopic analysis of the sophisticated olive oil [4]. Since the amount of pigments is a distinct feature of EVOOs, with respect to other oils, the identification and quantification of pigments have become the subject of intense research, and several methodologies are now available [5–15].

## 2. Carotenoids and chlorophyll derivatives

The unique colour of olive oil related to its pigment content [16] varies from a light gold to a rich green. Green olives produce a green oil because of the high chlorophyll content, while ripe olives yield a yellow oil because of the carotenoids (yellow red). The exact combination and proportions of pigments determine the final colour of the oil. Their presence in olive oil depends on olive fruits (*Olea europa, L.*), but also on genetic factors (olive cultivar), the stage of fruit ripeness, environmental conditions, the extraction processing [8, 17–19] and storage conditions. The role of chlorophylls as natural pigments accounting for greenish colours and in photosynthesis is well known. There are also some reports about the benefits of dietary chlorophylls for human health [20].

The structure of chlorophyll pigments, consisting of one tetrapyrrole macrocycle, coordinated to a $Mg^{++}$ ion to form a planar complex, is responsible for the absorption in the visible region of the spectrum of olive oils. Here, both the bluish-green chlorophyll-a and the yellowish-green chlorophyll-b can be found. Chlorophylls in olive oils are mostly converted to pheophytins, due to the exchange of the central $Mg^{++}$ ion with acid protons. Pheophytin-a (see **Figure 1a**) is

predominant with respect to pheophytin-b. In the case of bad storage conditions, pheophytins are further degraded to pyropheophytins [21, 22]. The main carotenoids present in olive oils are lutein and β-carotene (see **Figures 1b**, **c**).

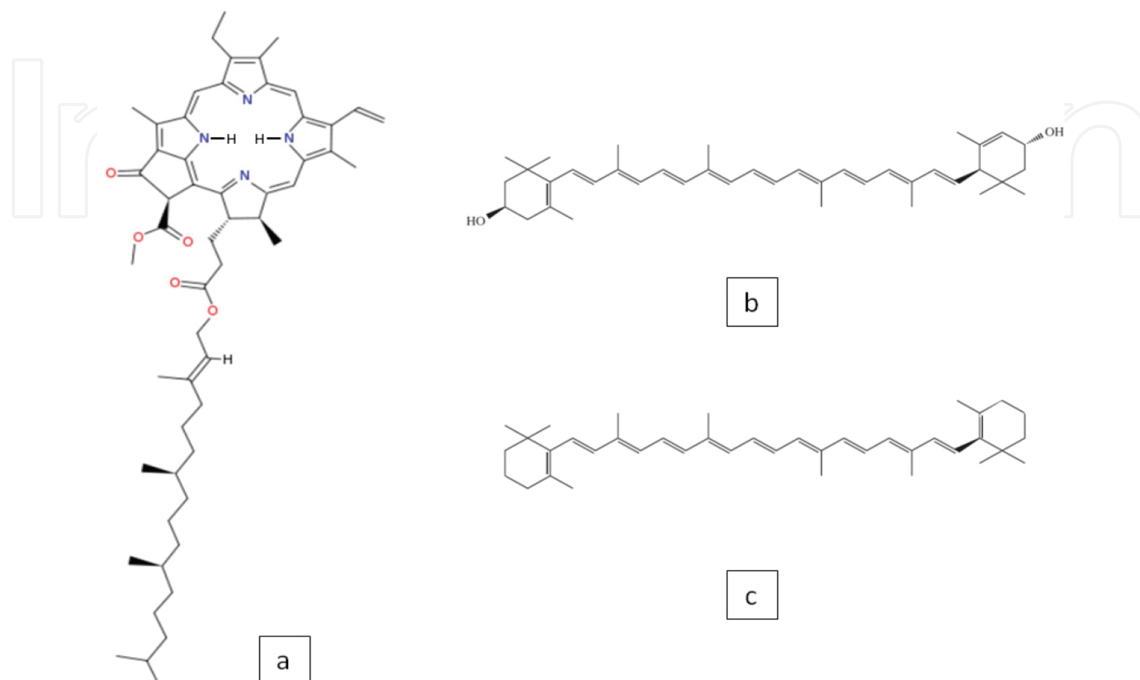


**Figure 1.** Molecular structure of (a) pheophytin-a, (b) lutein and (c) β-carotene.

The level of pigments ranges from a few ppm to almost 100 ppm. Fresh olive oils usually have a higher pigments content. The major components are pheophytin-a (from few ppm up to 25 ppm), followed by β-carotene (from few ppm up to 15 ppm) and lutein (from few ppm up to 10 ppm).

Carotenoids are isoprenoid compounds having a hydrocarbon structure with carbon double bonds that are responsible for many of their properties [23, 24]. Carotenoids can be divided into carotenes (carotenoids containing only carbon and hydrogen) and xanthophylls (carotenoids that also contain oxygenated functions, such as epoxide, hydroxyl, acetate, carbonyl and carboxylic groups, among others). In EVOOs, the main carotenoids are β-carotene and lutein [25]. The carotenoid fraction of olive oil also includes other xantophylls [15, 16].

See also Chapter entitled "Chlorophylls and Carotenoids in Food Products from Olive Tree" by Beatriz Gandul-Rojas, María Roca and Lourdes Gallardo-Guerrero.

## 3. Methods to identify and quantify pigments in olive oils

In the literature, several works have been published about analytical methods able to identify and quantify pigments in oil matrices. These methods can be divided into two main categories

based on the physical principle: (1) chromatographic techniques (characterized by pretreatment of the samples, such as extraction and/or saponification) and (2) spectroscopic techniques (without pretreatment of the samples). In the following, these two main classes of methods are overviewed.

### 3.1. Chromatographic techniques

The most successful chromatographic technique for pigments quantification is the *high-performance liquid chromatography,* HPLC, coupled with mass spectroscopy or UV-vis absorp-

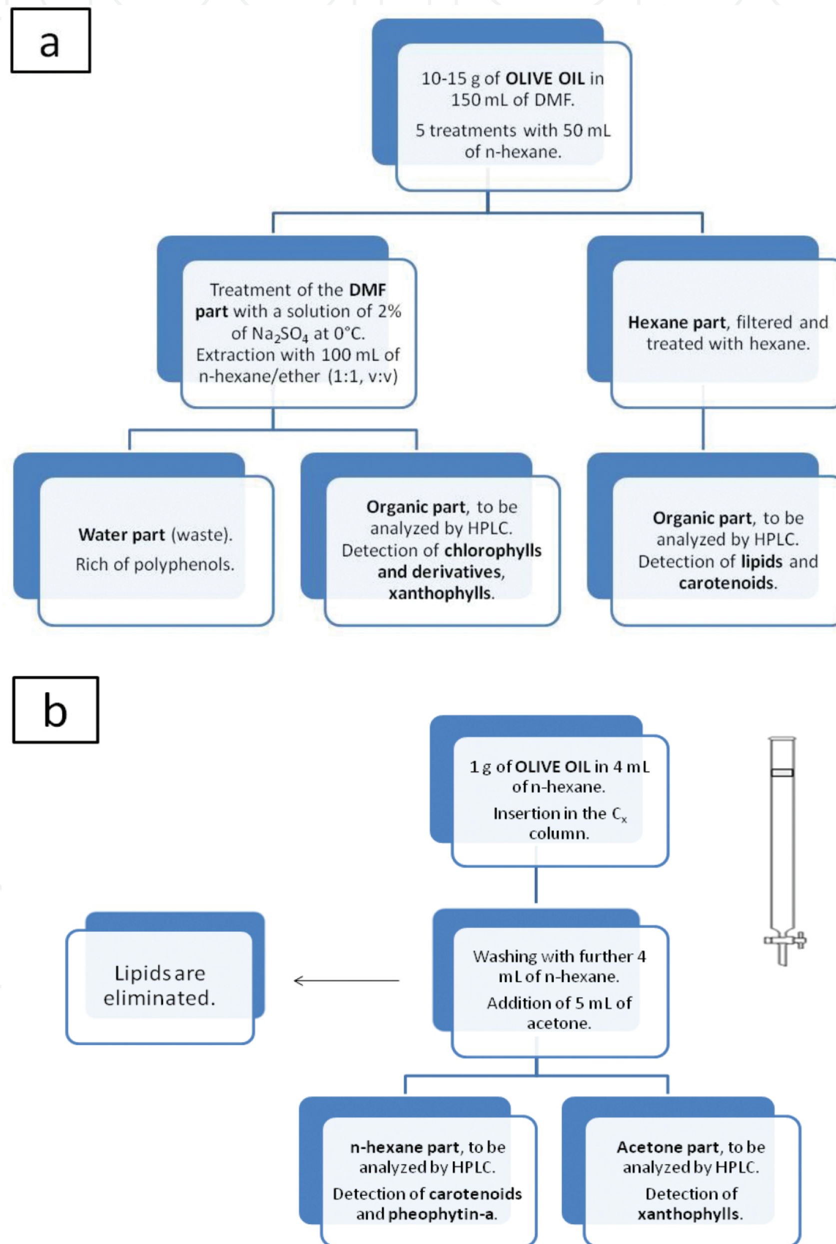


**Figure 2.** Basic treatment schemes to analyse the pigments' content in EVOOs according to (a) the (LPD) [26] and (b) the SPE [27] chromatographic methods.

tion through the diode array detection (DAD). These methods consist of three steps: (a) extraction/purification of the pigments from olive oil sample; (b) chromatographic separation of pigments; and (c) fraction analysis. The extraction/purification step can be done by *liquid phase distribution* (LPD) [26] or by *solid phase extraction* (SPE) [27]. The LPD method includes the separation of pigments between two phases, one in hexane and the other one in N,N-dimethyl formaldehyde (DMF), as shown in **Figure 2a**. On the contrary, the SPE method is based on the use of $C_{18}$ columns, which are previously conditioned with a mixture of methanol and hexane. Details of the procedure are reported in **Figure 2b**. The comparison between the two procedures shows that the recoveries of lutein, β-carotene and pheophytin-a are much better in the SPE than LPD method [28]. The chromatographic separation of pigments is highly dependent on the column: direct columns are normally very sensitive to the eventual presence of water and temperature gradients, and they require longer retention times [29]. Inverse column gives better results. The use of $C_{18}$ inverse column allows optimal separation of chlorophylls and their derivatives, as well as separation of short chain and low molecular weight carotenoids [30].

The recent introduction of $C_{30}$ inverse columns allowed a good separation for long chain carotenoids and their isomers with lower polarity, such as lycopene and β-carotene [15, 31]. A disadvantage of $C_{30}$, with respect to $C_{18}$, is the longer retention times. Normally, the better choice of the column depends on the starting matrix [32-34].

Solvent mixtures to analyse an oil matrix have been optimized [33], and they consist of eluent A, based on water/reagent ion pair/methanol (1:1:8, v/v), and eluent B, based on acetone/methanol (1:1, v/v). The reagent *ion pair* is represented by a solution of tetrabutylammonium (0.05 M) and ammonium acetate (1 M).

| | **Mobile phase** | |
|---|---|---|
| **Time (min)** | **A (%)** | **B (%)** |
| 0 | 75 | 25 |
| 8 | 25 | 75 |
| 10 | 25 | 75 |
| 18 | 10 | 90 |
| 23 | 0 | 100 |
| 30 | 75 | 25 |

**Table 1.** Elution gradient for pigments separation commonly used in HPLC methods.

The gradient scheme used for pigments separation is reported in **Table 1**.

Mass spectroscopy (MS) has been used less frequently to analyse pigments in olive oils. This technique is very useful to understand the structural features and degradation of chlorophylls

due to oxidative effects. Recently, a new methodology based on HPLC coupled with high-resolution time-of-flight (hrTOF) mass spectrometry has been developed, and thanks to the computer-assisted analysis, the completion of MS fragmentation of chlorophylls and their derivatives has been reached [35]. No applications of this technique in terms of authentication and quality aspects of olive oils have been reported so far.

| **Pigments** | **k** | **Peak positions (nm)** | | | **Ratio between peaks** | |
|---|---|---|---|---|---|---|
| | | **I** | **II** | **III** | **100 III/II** | **I/II** |
| Neoxanthin | 2.4 | 415 | 438 | 467 | 88 | – |
| Neoxanthin | 2.8 | 415 | 438 | 467 | 88 | – |
| Violaxanthin | 3.1 | 413 | 434 | 464 | 93 | – |
| Luteoxanthin | 3.4 | 398 | 420 | 447 | 100 | – |
| Auroxanthin | 3.6 | 379 | 400 | 425 | 94 | – |
| Antheraxanthin | 4.1 | 421 | 445 | 470 | 45 | – |
| Mutatoxanthin | 4.4 | 414 | 438 | 464 | 60 | – |
| Auroxanthin isomer | 4.7 | 379 | 400 | 425 | 92 | – |
| Chlorophyll-b | 4.8 | 465 | 602 | 650 | – | 3.3 |
| Lutein | 5.1 | 423 | 444 | 472 | 63 | – |
| b-Cryptoxanthin | 5.8 | 431 | 450 | 477 | 28 | – |
| cis-Lutein | 6.3 | 416 | 438 | 466 | 38 | – |
| Chlorophyll-a | 6.7 | 430 | 620 | 666 | – | 1.1 |
| Neoxanthin | 7.3 | 415 | 438 | 467 | 88 | – |
| Pyropheophytin-a | 9.7 | 406 | 506 | 666 | – | 2.2 |
| Pheophytin-a′ | 12.1 | 406 | 506 | 665 | – | 2.1 |
| Pheophytin-a | 12.4 | 406 | 506 | 665 | – | 2.1 |
| Pheophytin-a isomer | 12.7 | 406 | 506 | 665 | – | 2.1 |

This table is modified from **Table 2** in Ref. [33] (reproduced with permission from Wiley Company).

**Table 2.** Parameters commonly used for the HPLC-DAD detection of pigments in EVOOs. The retention factor, k, the peak positions of the UV-vis spectra for DAD detection and the ratios between relevant peaks are reported (see reference 33).

### 3.2. Spectroscopic techniques

Spectroscopic techniques, such as UV-vis absorption, nuclear magnetic resonance (NMR), Fourier transform infrared (FT-IR) spectroscopy and fluorescence, are used to investigate olive oil chemical composition and to differentiate oils according to cultivar, variety and storage conditions. The main feature of these techniques is the possibility to use them directly on olive oil samples, without any pretreatment. In most of the cases, these techniques are suitable for

investigating the saponifiable fraction of olive oil, as is the case of FT-IR [36], and NMR spectroscopy [37, 38].

The broadness of the electromagnetic spectrum allows to get information from different spectral regions; those ones mostly used to study food and relative frauds are three: (1) the ultraviolet (UV) region (200–400 nm), (2) the visible (Vis) region (400–779 nm) and (3) the near-infrared (NIR) region (780–2500 nm).

In particular, the UV region is characterized by high signal to noise ratio and high sensitivity, due to the presence of many signals from tocopherols, anthocyanins, phenols and diene/triene compounds [39].

As it is shown in **Figure 3**, the UV region is characterized by very intense signals in extra-virgin olive oils, due to the variety of minor components.

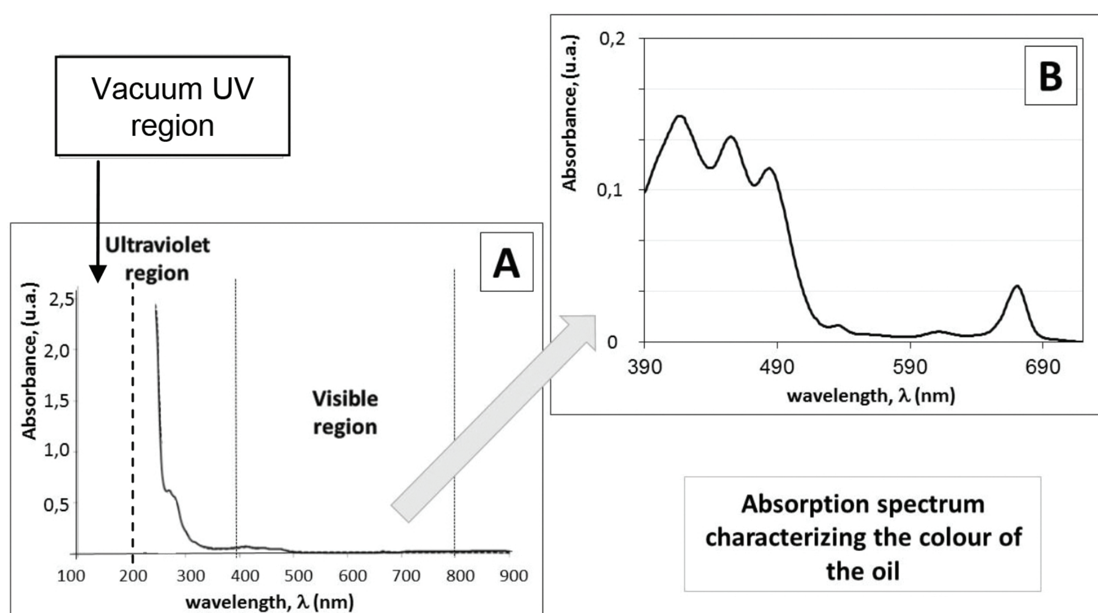


**Figure 3.** (A) Full experimental spectrum in the UV-vis region of an extra-virgin olive oil, showing the two separated regions: UV and Vis one. (B) Enlargement of the spectrum in the sole Vis region showing the typical shape of the Vis absorption spectrum of extra-virgin olive oils, due to the pigments content and responsible of the colour.

Concerning the NIR absorption region, most of the applications on olive oils concern the acidic part, allowing a differentiation among different botanic origin of seeds oils [40], but with this technique, pigments cannot be investigated.

A useful technique to study chlorophylls and carotenoids is fluorescence spectroscopy [41], which is a photoluminescence process. Fluorescence spectroscopy can be performed either by right-angle and front-face techniques, with several possibilities of investigation. Different types of signals can be indeed acquired: emission spectra, excitation spectra and synchronous spectra [42]. Several works have been published based on fluorescence spectroscopy applied to extra-virgin olive oils [43–49], since several chemical constituents, including pigments, give fluorescence in specific conditions and they can be identified. The quantification of fluoro-

phores is less straightforward than in absorption spectroscopies, as deeply addressed in Ref. [49]. In particular, this work shows that the right-angle method gives several artefacts, while front face technique is more appropriate for quantification of fluorophores. In emission spectra of olive oils, the band centred at 670–680 nm is clearly due to chlorophyll-like chromophores. In particular, the pheophytins' signals are expected around 670 nm, but the maximum of the emission band can be slightly shifted depending on the used technique (right-angle or front-face). Interestingly, a weak emission band, centred at about 666 nm in the spectrum of sunflower oil, reveals the presence of fluorescent chlorophyll derivatives. Such a band is undetectable in peanut oil [49]. Carotenoids, present in olive oil with a relatively high concentration, are characterized by a less intense emission. In fact, carotenoids strongly compete for incident light with other chromophores present in olive oil due to their concentration and high extinction coefficient. Carotenoids signals can be observed in the 430–480 nm region, but their quantification remains very difficult by fluorescence techniques.

The synchronous fluorescence technique has been developed to analyse large sets of olive oil samples with the aim of differentiate them on the basis of botanic origin. This technique consists in acquiring emission and excitation spectra simultaneously, by fixing a constant distance between the emission and excitation wavelengths [45–48]. This spectroscopic method is coupled with a multivariate statistical analysis, such as PLS, *partial least squares*, PCA, *principal components analysis*, or PARAFAC, *PARAllel FACtor analysis*. These methods have been proved to be satisfactory for discriminating oils of different botanic origin based on chlorophylls and their derivatives quantification, but not for carotenoids. Nevertheless, despite of good potentialities, no works are known about applications in the field of frauds and authentication of EVOOs.

### *3.2.1. Methods based on Vis absorption for pigments identification and quantification*

As previously stated, Vis absorption spectra of extra-virgin olive oils have characteristic features [50, 51]: a three-peak band in the range 390–520 nm and a sharper band around 660–675 nm. This last absorption band is due to the electronic transition of chlorophylls and their derivatives, while the first band is more complex, since it is due to the overlap among carotenoids and chlorophylls absorption signals. As it can been seen in **Figure 4**, the visible absorption spectrum of extra-virgin olive oils is very different from the absorption spectra of other seeds and fruits oils. This evidence is at the basis of several works [5, 11, 52–54] devoted to the authentication of EVOOs and the identification of specific frauds, such as the mixing between olive oils and oils obtained from other seeds.

Visible (vis) light absorption of EVOOs is associated with the pigments' content, and this specificity is at the origin of several research works aiming to substitute the chromatographic methods with much faster and direct spectrophotometric methods [5, 11, 12, 39].

A recent methodology proposed by Cayuela et al. [12] associates the absorbance measured at specific wavelengths in the visible region, namely the K470 and K670 indexes, to the amount of carotenoids and chlorophyll derivatives, respectively. This method has the advantages to be fast, non-destructive and inexpensive. However, the single absorbance values at specific wavelengths in the Vis spectrum of EVOOs do not allow a reliable and

unambiguous quantification of the pigments' content, in particular for the superposition of carotenoids and pheophytins signals in the 390–520 nm region. Moreover, this method is able to quantify only the total amount of carotenoids and chlorophyll derivatives, and not the single compounds.

In order to get a more robust and accurate method for pigments quantification, fast and rapid at the same time, a mathematical approach based on the spectral deconvolution of the Vis absorption of EVOOs in terms of its main pigment components can be performed. A first mathematical approach was proposed by Ayuso et al. [50]: the experimental Vis spectrum is reproduced by a combination of two signals, from pure β-carotene and chlorophyll-a spectra. More recently, a new mathematical approach has been developed by using four orthogonal functions derived from the experimental spectra of two carotenoids (β-carotene and lutein) and two chlorophyll derivatives (pheophytin-a and pheophytin-b). This procedure, shown in **Figure 5**, consists of the following steps: (1) the acquisition of the experimental UV-vis spectrum of the sample; (2) the fitting of the experimental spectrum as a linear combination of the four orthogonal functions; and (3) the calculation of pigments' concentrations and relevant statistical parameters. Steps 2 and 3 are done automatically by a home-made program compatible with Excel software [5].

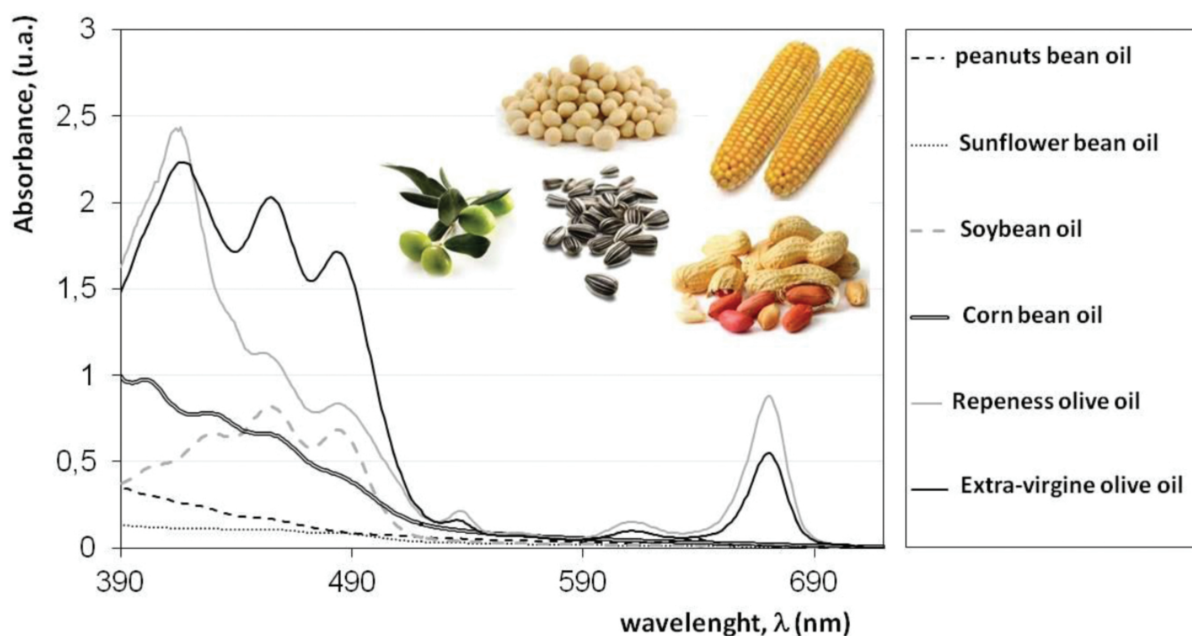


**Figure 4.** Visible absorption spectra of several vegetable oils obtained from olives, peanuts, sunflower beans, soya beans and corn beans.

This method has been applied to more than 150 EVOOs having different cultivars and with different geographic origin [5, 51, 52, 54], showing a very high quality of the fitting in all cases ($R^2$ ranges from 0.996 to 0.998) and good possibilities of discriminating EVOOs from not EVOOs.

Additional methods to analyse olive oils from UV-vis absorption spectra base on statistical multivariate approaches. In such cases, the discriminating factors are derived from the absorption spectra, but they are not directly related to the pigments concentration. An example is the method based on the calculation of chaotic parameters (Lyapunov exponent, autocorrelation coefficients and fractal dimensions), further treated to find correlations index [10, 11, 55] and the application of PCA on large sets of data, including UV-vis spectral ones, further treated with class-modelling methods [56].

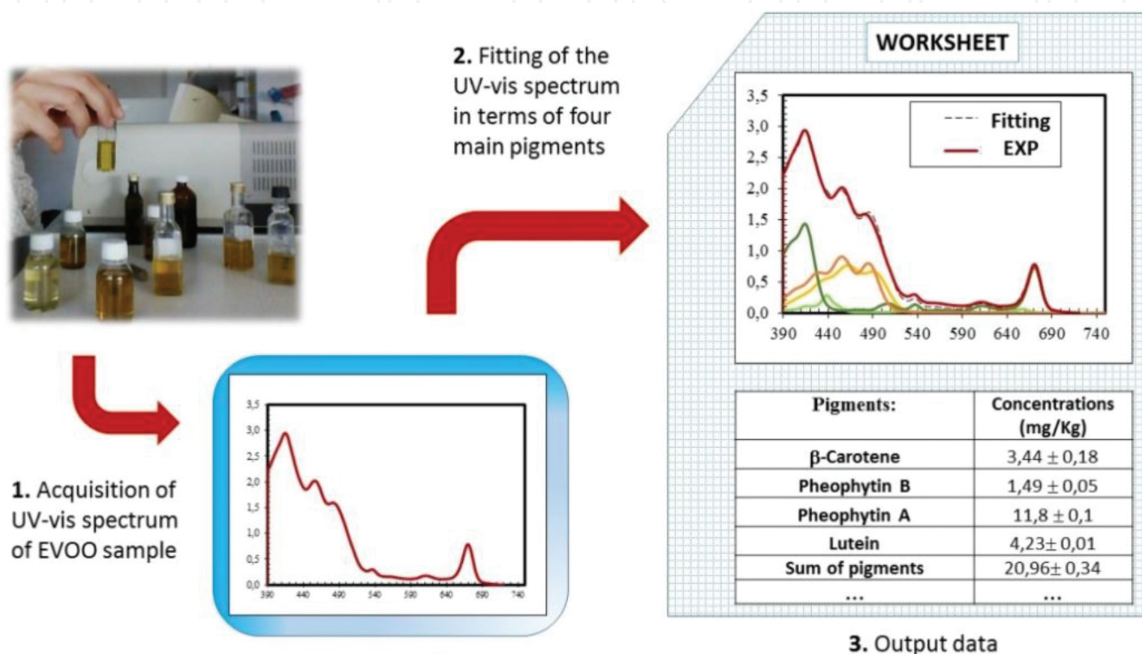


**Figure 5.** Scheme of the mathematical method develop [5] to obtain the concentration of four main pigments by analysing the Near UV-vis absorption spectrum of an EVOO.

## 4. Authenticity and quality studies based on pigments quantification

As seen in the previous sections, there are several analytical methods, both chromatographic and spectroscopic ones, able to identify and quantify pigments in EVOOs. Since the concentration of pigments strongly differs depending on several variables, their use was discouraged. It is well known that chlorophylls completely degrade into pheophytins after few months after the bottling and that the pheophytins themselves evolve into pyropheophytins depending on storage conditions. However, as reported in several works [5, 51–60], typical range of concentrations of various pigments can be associated with EVOOs and these quantities can be used to discriminate them from non-EVOOs. Most of the applications of the chromatographic methods refer to Spanish EVOOs [56–58], while the recently developed UV-vis based mathematical approach [5] has been mainly applied to Italian [5, 51, 52, 54] and Spanish EVOOs [5, 53] as well as Greek and Tunisian EVOOs [53, 54].

http://dx.doi.org/10.5772/64736

The analytical methodologies described in the previous section can be roughly divided into two types: methods able to characterize the olive varieties and cultivars and methods developed to identify the geographic origin of the olives. Some parameters used for EVOOs authentication are the ratio between the total amount of chlorophylls' derivatives and the total amount of carotenoids, the ratio between the carotenoids and lutein contents, the percentage of violaxhantin and the percentage of lutein [57–59].

For instance, according to Ref. [56], Spanish EVOOs typically have a ratio between chlorophylls' derivatives and carotenoids close to 1, in case of Italian EVOOs, a higher variability of this ratio has been found [15, 49–52]. According to Ref. [57], the ratio between carotenoids and lutein varies in a range between 0.4 and 1.5, depending on the variety and cultivar and this factor could be used as authentication parameter. Another parameter to be taken into account for the authenticity of EVOOs is the ratio between the concentration of lutein and β-carotene, which typically varies between 0.15 and 5 [15, 53, 54, 60]. However, as also reported in Refs. [19, 54], the amount of pigments in EVOOs depend strongly by olives variety, by the moment when the olives are picked and by the storage conditions [13, 57, 59].

## 5. Conclusions

In this chapter, the main analytical methods developed and applied to the identification and quantification of pigments in edible oils, and in particular, extra-virgin olive oils are described. New methodologies, divided between chromatographic and spectroscopic ones, are discussed putting in evidence advantages and disadvantages.

Pigments' concentration and their relative ratios may indicate the age and storage conditions (i.e. temperature, light and oxygen exposure), but they are also a good parameter to check authenticity and quality and to reveal mixtures with other seed oils, as deeply addressed in this chapter.

## Acknowledgements

We would like to thank Dr. Andrea Serani and Prof. Maurizio Zandomeneghi for helpful discussions during our researches on olive oils.

## Author details

Cristina Lazzerini, Mario Cifelli and Valentina Domenici*

*Address all correspondence to: valentina.domenici@unipi.it

University of Pisa, Pisa, Italy

## References

[1] Spink J., Moyer D.C. Understanding and combating food fraud. Food Technology Magazine. 2013;67:30–35.

[2] Boskou D., Blekas G., Tsimidou M. Olive oil composition. In: Boskou D., editor. Olive Oil Chemistry and Technology. 2nd ed. Champain: AOCS Press; 2006. p. 40–72.

[3] Clydesdale F.M. Color as a factor in food choice. Critical Reviews in Food Science and Nutrition. 1993;33:83–101.

[4] Del Giovine L., Fabietti F. Copper chlorophyll in olive oils: identifications and determination by LIF capillary electrophoresis. Food Control. 2005;16:267–272.

[5] Domenici V., Ancora D., Cifelli M., Serani A., Veracini C.A., Zandomeneghi M. Extraction of pigment information from near UV-Vis absorption spectra of extra virgin olive oils. Journal of Agricultural and Food Chemistry. 2014;62:9317–9325.

[6] Serani A., Piacenti D. Analytical technique for the identification of deodorized oils in virgin olive oils. Note 1 – Analysis of chlorophylls' derivatives in virgin olive oils. The Italian Journal of Fatty Compounds. 2001;LXXVIII:459–463.

[7] Angerosa F., Campestre C., Giansante L. Analysis and authentication. In: Boskou D., editor. Olive Oil Chemistry and Technology. 2nd ed. Champain: AOCS Press; 2006. p. 113–172.

[8] Gandul-Rojas B., Roca-L., Cepero M., Mínguez-Mosquera M.I. Use of chlorophyll and carotenoid pigment composition to determine authenticity of virgin olive oil. Journal of American Oil Chemists's Society. 2000;77:8.

[9] Moyano M.J., Melendez-Martinez A.J., Alba J., Heredia F.J. A comprehensive study of the colour of the virgin olive oils and its relationship with the chlorophylls and carotenoids indexes (I): CIEXYZ non-uniform colour space. Food Research International. 2008;41:505–512.

[10] Torrecilla J.S., Rojo E., Domínguez J.C., Rodríguez F. Linear and non linear chemometric models to quantify the adulteration of extra virgin olive oil. Talanta. 2010;83:404–409.

[11] Torrecilla J.S., Rojo E., Dominguez J.C., Rodriguez F. A novel method to quantify the adulteration of extra-virgin olive oil with low grade olive oils by UV-Vis. Journal of Agricultural Food Chemistry. 2010;58:1679–1684.

[12] Cayuela J.A., Yousfi K., Martinez M.C., Garcia J.M. Rapid determination of olive oil chlorophylls and carotenoids by using UV-Vis spectroscopy. Journal of American Oil Chemists's Society. 2014;91:1677–1684.

[13] Psomiadou E., Tsimidou M. Simultaneous HPLC determination of tocopherols, carotenoids and chlorophylls for monitoring their effect on virgin olive oil oxidation. Journal of Agricultural and Food Chemistry. 1998;46:5132–5138.

[14] Seppanem C.M., Rahmani M., Csallany A. Simultaneous determination of chlorophylls, pheophytins, b-carotene, tocopherols and tocotrienols in olive and soybean oils by HPLC. Journal of Food Science. 2003;68:1644–1647.

[15] Giuffrida D., Salvo F., Salvo A., Cossignani L., Dugo G. Pigments profile in monovarietal virgin olive oils from various Italian olive varieties. Food Chemistry. 2011;124:1119–1123.

[16] Minguez-Mosquera I., Gandul B., Garrido J., Gallardo L. Pigments present in virgin olive oils. Journal of American Oil Chemists's Society. 1990;67:192–196.

[17] Giuliani A. Chlorophylls in olive and in olive oil: chemistry and occurrences. Critical Reviews in Food Science and Nutrition. 2011;51:678–690.

[18] Ramirez E., Gandul-Rojas B., Romero C., Brenes M., Guerrero L.G. Composition of pigments and colour changes in green table olives related to processing type. Food Chemistry. 2015;66:115–124.

[19] Criado M.N., Romero M.P., Motilva M.I. Effect of the technological and agronomical factors on pigment transfer during olive oil extraction. Journal of Agricultural and Food Chemistry. 2007;55:5681–5688.

[20] Ferruzzi M.G., Blakeslee J. Digestion, absorption and cancer preventative activity of dietary chlorophyll derivatives. Nutrition Research. 2007;27:1–12.

[21] Gertz C., Fiebing H.J. Pyropheophytin a determination of thermal degradation products of chlorophyll a in virgin olive oil. European Journal of Lipid Science and Technology. 2006;108:1062–1065.

[22] Aparicio R. Thermal degradation kinetics of chlorophyll pigments in virgin olive oils, A. Compounds of series A. Journal of Agricultural and Food Chemistry. 2010;58:6200–6208.

[23] Britton G. Structure and properties of carotenoids in relation to functions. The Federation of American Societies for Experimental Biology Journal. 1995;9:1551–1558.

[24] Mendez-Martinez A.J., Vicario I.M., Heredia F.J. Carotenoid pigments: structural and physicochemical considerations. Latin-American Archive of Nutrition. 2007;57:109–117.

[25] Jaswir I., Noviendri D., Rewo F.H., Octavianti F. Carotenoids: sources, medical properties and their application in food and nutraceutical industry. Journal of Medical Plants Research. 2011;71:7119–7131.

[26] Minguez-Mosquera M.I., Garrido-Fernandez J. Chlorophyll and carotenoid presence in olive fruit (Olea europeae). Journal of Agricultural and Food Chemistry. 1989;37:1–7.

[27] Gutierrez F., Albi M.A., Palma R., Rios J.L., Olias J.M. Bitter taste of virgin oil: correlation of sensory evaluation and instrumental HPLC. Journal of Food Science. 1989;54:68–73.

[28] Minguez-Mosquera M.I., Gandul-Rojas B., Gallardo-Guerrero M.L. Rapid method of quantification of chlorophylls and carotenoids in virgin olive oils by HPLC. Journal of Agricultural and Food Chemistry. 1992;40:60–63.

[29] Schmitz H.H., Emenhiser C., Schartz J.J. HPLC separation of geometric carotene isomers using a calcium hydroxide stationary phase. Journal of Agricultural and Food Chemistry. 1995;43:1212–1218.

[30] Kamal-Eldin A. Minor components of fats and oils. Bailey's Industrial Oil and Fat Products. 2005;3:12.

[31] Giuffrida D., Salvo F., Salvo A., La Pera L., Dugo G. Pigments composition in monovarietal virgin olive oils from various sicilian varieties. Food Chemistry. 2007;101:833–837.

[32] Crupi P., Milella R.A., Antonacci D. Simultaneous HPLC-DAD-MS ($ESI^+$) determination of structural and geometrical isomers of carotenoids in mature grapes. Journal of Mass Spectrometry. 2010;45:971–980.

[33] Gandul-Rojas B., Mìnguez-Mosquera M.I. Chlorophyll and carotenoid composition in virgin olive oils from various Spanish olive varieties. Journal of the Science of Food and Agriculture. 1996;72:31–39.

[34] Criado M.N., Motilva M.J., Gòni M., Romero M.P. Comparative study of the effect of the maturation process of the olive fruit on the chlorophyll and carotenoid fractions of drupes and virgin oils from Aberquina and Farga cultivars. Food Chemistry. 2007;100:748–755.

[35] Chen K., Rìos J.J., Pèrez-Gàlvez A., Roca M. Development of an accurate and high-throughput methodology for structural comprehension of chlorophylls derivatives (I). Dephytylated derivatives. Journal of Chromatography A. 2015;14:99–108.

[36] Baeten V., Dardenne P., Aparicio R. Interpretation of Fourier transform Raman spectra of the unsaponifiable matter in a selection of edible oils. Journal of Agricultural and Food Chemistry. 2001;49:5098–5107.

[37] Dais P., Hatzakis E. Quality assessment and authentication of virgin olive oil by NMR spectroscopy: a critical review. Analytica Chimica Acta. 2013;765:1–27.

[38] Del Coco L., Schena F.P., Fanizzi F.P. $^1H$ nuclear magnetic resonance study of olive oils commercially available as Italian products in the United States of America. Nutrients. 2012;4:343–355.

[39] Pizarro C., Rodrìguez-Tecedor S., Pèrez-del Notario N., Esteban-Dìez I., Gonzàlez-Sàiz J.M. Classification of Spanish extra virgin olive oils by data fusion of visible spectroscopic fingerprints and chemical descriptors. Food Chemistry. 2013;138:915–922.

[40] Mignani A.G., Garcìa-Allende P.B., Ciaccheri L., Conde M.O., Cimato A., Attilio C., Tura D. Comparative analysis of quality parameters of Italian extra virgine olive oils according to their region of origin. Optical Sensor. 2008;70:1–6.

[41] Sikorska E., Khmelinskii I., Sikorski M. Analysis of olive oils by fluorescence spectroscopy: methods and applications. In: Boskou D., editor. Olive Oil-Constituents, Quality, Health Properties and Bioconversions. Rijeka; InTech; 2012. p. 63–89.

[42] Lakowicz J.R. Principles of Fluorescence Spectroscopy. 3rd ed. New York: KluwerAcademic/Plenum Publishers; 2006.

[43] Young A.J., Frank H.A. Energy transfer reactions involving carotenoids: quenching of chlorophyll fluorescence. Journal of Photochemistry and Photobiology. 1996;36:3–15.

[44] Choe E., Min D.B. Mechanisms and factors for edible oils oxidation. Comprehensive Reviews in Food Science and Food Safety. 2006;5:169–186.

[45] Guimet F., Boque R., Ferre J. Cluster analysis applied to the exploratory analysis of commercial Spanish olive oils by means of excitation-emission fluorescence spectroscopy. Journal of Agricultural and Food Chemistry. 2004;52:6673–6679.

[46] Poulli K.I., Mousdis G.A., Georgiou C.A. Classification of edible and lampante virgin olive oil based on synchronous fluorescence and total luminescence spectroscopy. Analytica Chimica Acta. 2005;542:151–156.

[47] Dìaz G.T., Meràs I.D., Correa C.A., Roldan B. Simultaneous fluorometric determination of chlorophylls a and b and Pheophytins a and b in olive oil by partial least-squares calibration. Journal of Agricultural and Food Chemistry. 2003;51:6934–6940.

[48] Moberg L., Robertsson G., Karlberg B. Spectrofluorimetric determination of chlorophylls and pheopigments using parallel factor analysis. Talanta. 2001;54:161–170.

[49] Zandomeneghi M., Carbonaro L., Caffarata C. Fluorescence of vegetable oils: olive oils. Journal of Agricultural and Food Chemistry. 2005;53:759–766.

[50] Ayuso J., Haro M.R., Escolar D. Simulation of the visible spectra for edible virgin olive oils: potential uses. Applied Spectroscopy. 2004;58:474–480.

[51] Ancora D., Domenici V. (tutor). UV-Vis and $^{1}$H-NMR spectroscopic methods applied to the study of extra-virgin olive oil produced in Tuscany and Apulia. Master Thesis, University of Pisa; 2014.

[52] Domenici V., Lazzerini C., Cifelli M. A rapid method to evaluate extra virgin olive oils quality from near-UV Vis absorption spectral analysis. Presented at 106th AOCS annual meeting, May 3, 2015 Orlando, USA.

[53] Lazzerini C., Cifelli M., Domenici V. Italian Journal of Food Science, under review.

[54] Lazzerini C., Buti F., Cifelli M., Zandomeneghi M., Domenici V. Tuscan Extra-virgin Olive Oil: a study of the pigments' content and perspectives for a new quality index. In: Lenzi A., editor. Codice Armonico 2016, Pisa: ETS; 2016.

[55] Torrecilla J.S., Vidal S., Aroca-Santos R., Wang S.C., Cancilla J. Spectroscopic determination of the photodegradation of monovarietal extravirgin olive oils and their binary mixtures through intelligent systems. Talanta. 2015;144:363–368.

[56] Oliveri P., Casale M., Casolino M.C., Baldo M.A., Nizzi F., Forina G.F. Comparison between classical and innovative class-modelling techniques for the characterisation of a PDO olive oil. Anal Bioanal Chem. 2011;399:2105–2113.

[57] Guerrero L.G., Gandul-Rojas B., Roca M., Minguez-Mosquera I. Effect of storage on the original pigment profile of Spanish virgin olive oil. Journal of the American Oil Chemists' Society. 2005;82:33–39.

[58] Gandul-Rojas B., Roca B.M., Cepero L., Minguez-Mosquera M.I. Chlorophylls and carotenoids pattern in olive fruits: Olea europea cv. Aberquina. Journal of Agricultural and Food Chemistry. 1999;47:2207–2212.

[59] Roca M., Gandul-Rojas B., Guerrero L.G., Minguez-Mosquera M.I. Pigments parameters determining Spanish virgin olive oil authenticity: stability during storage. Journal American Oil Chemists's Society. 2003;80:1237–1240.

[60] Roca M., Minguez-Mosquera M.I. Change in the natural ratio between chlorophylls and carotenoids in olive fruit during processing for virgine olive oil. Journal of the American Oil Chemists Society. 2001;78:133–138.

IntechOpen

IntechOpen