COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

The Color of Olive Oils: The Pigments and Their Likely Health Benefits and Visual and Instrumental Methods of Analysis

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ABSTRACT: The color of olive oils, and of foods in general, can influence consumer choices to a large extent and can be related to the processing treatments they have undergone. Olive oil color is due to 2 types of pigments, chlorophylls and carotenoids, which are attracting the attention of the scientific community due to the probable health benefits they can provide. Appropriate methodologies for the meaningful definition of the color of olive oil are therefore necessary for various reasons. In this review, we discuss the importance of olive oil color and the applicable legislation and regulation, including sections devoted to the pigments accounting for the growing importance as likely health-promoting substances. Furthermore, we review in depth the different approaches (visual and instrumental methods) used for color measurements in the last 50 y. Instrumental methods have been shown to be highly appropriate for objective assessments and also for the rapid determination of the pigments.

The Importance of Olive Oil Color: Not Only a Matter of Acceptability

Olive oil is an important component of the praised Mediterranean diet, which is attracting increasingly the interest of scientists due to the health benefits it can provide (Visioli and Galli 2002; Pérez-Jiménez 2007; Pérez-Jiménez and others 2007). As is the case for foodstuffs in general (Francis 1995), the color of olive oil is very related to its perceived quality and therefore to its acceptability (Ranalli and others 1997; Boskou 1998), hence this parameter is being paid much attention in recent years, above all in Spain (Mínguez-Mosquera and others 1991; Escolar and others 1994, 2007; Moyano and others 1999, 2001, 2008a, 2008b; Melgosa and others 2000, 2001, 2004, 2005; Romero and others 2001; Criado and others 2008).

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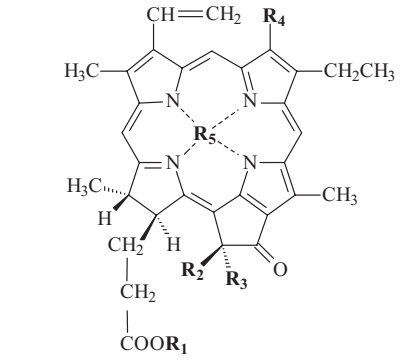
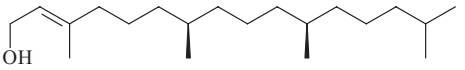
Color and acceptability

There is no doubt that, in the first place, consumers judge foods according to their external appearance (color, texture, and so on), so this 1st assessment is going to influence decisively their choices. This is partly due because the color of foods in general is frequently related to their stage of maturity, the presence of contaminants or microorganisms, the conditions of the industrial processing, and more. On the other hand, there are associations with food-appropriate colors, which are acquired mainly through learning; and they play an important role in the consumer choices (Clydesdale 1993). This link between the coloration of a given product and its acceptability has been long known by the food industry; hence, the use of colorants, either synthetic or natural, is so widespread.

According to all these facts, it can be claimed that the importance of olive oil color in its acceptability is such that the consumer can reject any given oil based on the appreciation of



Basic structure of some chlorophyll pigments



Phytol*

Figure 1 — Chemical structures of chlorophyll pigments.

Pigment	R_1	R_2	R_3	R_4	R_5	Color
Chlorophyll a	Phytol*	H	$COOCH_3$	CH_3	Mg^{2+}	Bluish-green
Chlorophyll b	Phytol*	H	$COOCH_3$	CHO	Mg^{2+}	Yellowish-green
Pheophytin a	Phytol*	H	$COOCH_3$	CH_3	2H	Brownish-grey
Pheophytin b	Phytol*	H	$COOCH_3$	CHO	2H	Olive green
Chlorophyllide a	H	H	$COOCH_3$	CH_3	Mg^{2+}	Bluish-green
Chlorophyllide b	H	H	$COOCH_3$	CHO	Mg^{2+}	Yellowish-green
Pheophorbide a	H	H	$COOCH_3$	CH_3	2H	Brownish-grey
Pheophorbide b	H	H	$COOCH_3$	CHO	2H	Olive green
Pyropheophytin a	Phytol*	H	H	CH_3	2H	Brownish-grey
Pyropheophytin b	Phytol*	H	H	CHO	2H	Olive green

*the hydroxyl group is lost when the phytol chain esterifies the propionic acid of the chlorophyll structure

its color, even though the rest of its quality parameters are appropriate. The derived economic importance of the appearance of the oils is therefore unquestionable. For instance, consumer surveys performed in the United Kingdom, a nontraditional market and therefore a potential new market for olive oil, revealed that there is a clear correlation between the acceptability data and the sensory attributes of the product. More specifically, positive correlations with color intensity and clarity and negative ones with lightness and green hue were observed (McEwan 1994).

Color as a useful tool in the food industry

The color parameters can also be very useful from a technological standpoint. As we will discuss latter, one of the main advantages of the objective measurement of color is that several parameters can be obtained in a matter of seconds; so, these color assessments can be very appropriate to obtain a rapid estimation of the effects of different commercial practices with olive oils. In fact, the chromatic intensities and pigment contents of olive oils have been already used as quality parameters when comparing different extraction methodologies. Overall, in these studies lower color intensities were related to higher qualities. More importantly, it was also concluded that the objective measurement of the intensities is basic to assess the quality of the product (Papasseit 1986; Ranalli 1992b; Di Giovachino and others 1994).

However, the interest in the color of olive oil, as that of many other products, goes beyond its relationship with consumer choices and commercial practices. This is because the pigments accounting for it (above all the carotenoids) are raising much interest due to their likely health benefits, as we will comment later on. Put in other words, it can be stated that the assessment of olive oil color can also be used for the rapid assessment of the levels of health-promoting compounds, which has great potential in relation to quality control in the food industry. In this regard, it is important to note that the application of multivariate statistical methods to correlate color parameters with pigment contents in olive oils (Mínguez-Mosquera and others 1991; Moyano and others 2008a, 2008b) and other foodstuffs is an expanding field (Arias and others 2000; Ronsholdt and McLean 2001; Meléndez-Martínez and others 2003, 2007a; Ruíz and others 2005, 2008). In this sense, we have reported recently that the color parameters can also be used for the rapid assessment of the vitamin A activity of orange juice (Meléndez-Martínez and others 2007b), which is due to only some of the carotenoid pigments occurring in it.

The Chemistry Behind the Color of Olive Oil

The color of olive oil is due to 2 types of natural pigments, chlorophylls and carotenoids (Mínguez-Mosquera 1997). Chlorophyll pigments account for the greenness of the oils, while the latter account for their yellowness. The structures of chlorophylls and carotenoids, many of which can be found in olive oils, are shown in Figure 1 and 2.

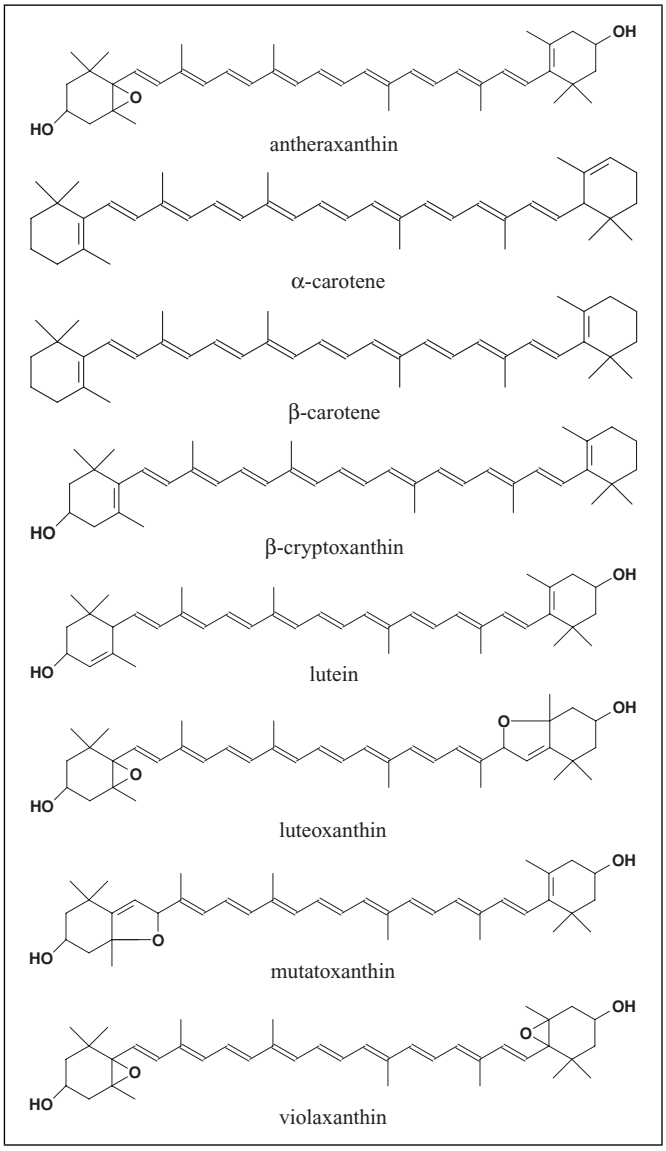


Figure 2—Chemical structures of some carotenoids reported in olive oils.

Chlorophyll pigments

The structure of chlorophyll pigments consists of one tetrapyrrole macrocycle (one of which is reduced), which contains an additional isocyclic ring. The macrocycle is coordinated to a Mg^{2+} to form a very stable planar complex (Figure 1). This structure contains a chromophore of several conjugated double bonds (CDBs) that is responsible for the absorption in the visible region of the spectrum of these pigments. In olive oils, both the bluish-green chlorophyll a and the yellowish–green chloprophyll b can be found. In some olive oils, the ratio between both pigments has been reported to oscillate between approximately 6 and 8 (Criado and others 2007b). The fundamental difference between them is that in the former, there is a methyl group in C_3, while in chlorophyll b there is a formyl group (Figure 1). The hydrophobic nature of these pigments is due to the presence of a molecule of phytol, which is esterified (R_1, Figure 1). Other chlorophyll pigments are pheophytins, chlorophyllides, pheophorbides, pyropheophytins, chlorines, rhodins, and purpurins (Mínguez-Mosquera 1997).

The pheophytins are formed as a consequence of the replacement of the magnesium ion in the chlorophyll molecules with 2 hydrogens. As a result of this change, pheophytin a exhibits a grey–brown color, whereas pheophytin b is olive green. On the other hand, the chlorophyllides are dephytilated derivatives of the chlorophylls that are formed by action of the enzyme chlorophyllase. Other chlorophyll derivatives, the pheophorbides, can be formed when the corresponding pheophytins are enzymatically dephytilated or when the chlorophyllides lose their magnesium cation in acidic medium. The pyropheophytins, in which the $COOCH_3$ group has been replaced by a hydrogen atom, are formed from the corresponding pheophytins as a result of prolonged heating. In other chlorophyll derivatives (chlorines, rhodins, and purpurins), the isocyclic ring is open. They can be formed as a result of oxidative processes in acidic or alkaline medium (Mínguez-Mosquera 1997).

The reactions that lead to the formation of these derivatives can occur when the cellular compartmentation of chlorophyll-containing tissues is lost and/or as a result of common senescence-related catabolic processes in the plant (Minguez-Mosquera and others 1993; Heaton and Marangoni 1996; Mínguez-Mosquera 1997; Hörtensteiner 2006; Roca and others 2007). As far as olives are concerned, there is no doubt that the crushing, milling, beating, heating processes, pH-changes, and so on they undergo to obtain oils and other products do favor the formation of chlorophyll derivatives. For instance, the main chlorophyll pigment in olive drupes is chlorophyll a, although during their processing to produce oil it gets in contact with acids and high quantities of its pheophytin a derivative are formed. The typical spectra of chlorophyll a and pheophytin a in methanol are shown in Figure 3. As it can be observed, chlorophyll a has 2 main absorption maxima located at 430 and 664 nm. In the case of pheophytin a, the 1st absorption maximum is located at a shorter wavelength (407 nm), and the intensity of the 2nd one is reduced, although it is located at a similar wavelength (666 nm). Visually, the transformation of chlorophyll a into its pheophytine derivative involves a shift of color from greenish hues to brownish hues.

Carotenoid pigments

Carotenoids are isoprenoid compounds that have a hydrocarbon structure with CDBs (Figure 2) that accounts for many of their properties and the actions they are involved in (Britton 1995a, 1995b). Most of the carotenoids described have 40 carbon atoms, although there are also carotenoids with shorter and longer structures. Depending on the presence or not of rings in their molecules, they can be classified into cyclic or acyclic carotenoids. Likewise, they can be divided into carotenes (carotenoids containing only carbon and hydrogen) and xanthophylls (carotenoids that also contain oxygenated functions, like epoxide, hydroxyl, acetate, carbonyl, and carboxylic groups, among others). In any case, carotenoids in natural structures can be free or associated with other compounds, such as fatty acids, sugars, and proteins (Britton and others 1995; Mínguez-Mosquera 1997; Rodriguez-Amaya 2001; Meléndez-Martínez and others 2007c).

The color of carotenoids is owed to the chromophore of CDBs, where the delocalization of the electrons is very high. When organic molecules absorb light, electronic transitions leading to an excited state of higher energy take place. In the case of carotenoids these transitions are from orbitals π to orbitals π^* and the excited state is of low energy, due to the delocalization of the electrons along the CDB system, such that the energy of visible light is enough to cause the electronic transitions (Britton

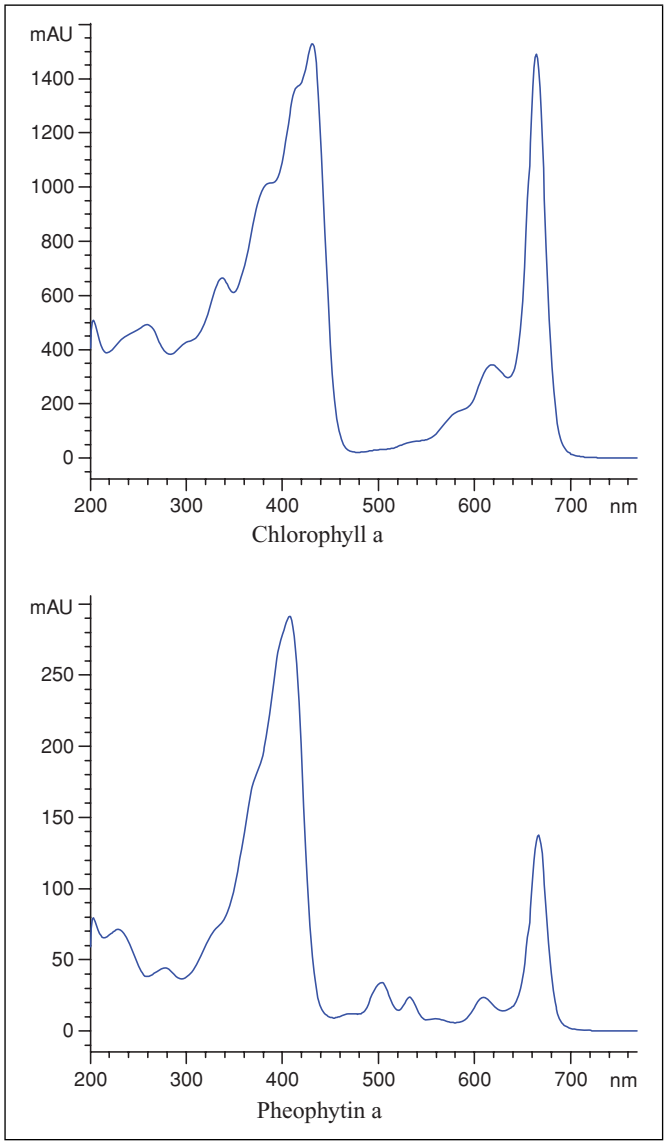


Figure 3—Spectra of chlorophyll a and pheophytin a in methanol.

1995b). Phytoene and phytofluene have 3 and 5 CDBs, respectively, and are colorless. Those carotenoids with 7 or more CDBs absorb light maximally between 400 and 500 nm, hence they furnish the yellowish, orange, and reddish colors of many structures (Britton 1995b; Rodriguez-Amaya 2001). The influence of the number and arrangement of CDBs in the absorption spectra of carotenoids has been long known. Recently, this spectroscopic information has been translated into color parameters by considering the CIELAB uniform color space (Meléndez-Martínez and others 2007). In the CIELAB color space (CIE 1978), 2 color coordinates, a^* and b^*, and a psychometric index of lightness, L^*, are defined. a^* is positive for reddish colors and negative for the greenish ones. The coordinate b^* is positive for yellowish colors and negative for the bluish ones. L^* is an estimation of the luminosity and allows to regard any given color as equivalent to a member of the gray scale, between black ($L^* = 0$) and white ($L^* = 100$). From a^* and b^*, the psychological parameters chroma

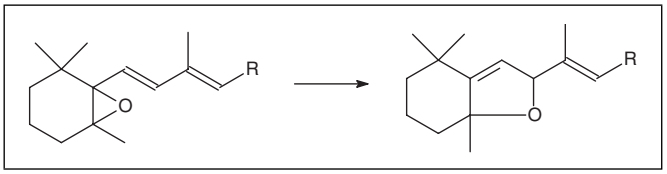


Figure 4—Scheme of the isomerization of 5,6-epoxides into 5,8-furanoids.

(C^*_{ab}) and hue (h_{ab}) are defined as

$$C^*_{ab} = [(a^*)^2 + (b^*)^2]^{1/2} \quad h_{ab} = \arctan(b^*/a^*)$$

C^*_{ab} allows to determine for each hue its degree of difference in comparison to a gray color with the same lightness. Chroma is considered as the quantitative attribute of colorfulness. The angular parameter hue (h_{ab}) is regarded as the qualitative attribute of color and, according to it, colors can be defined as reddish, greenish, and so on. More specifically, it is the attribute that allows to distinguish a color from a gray color with the same lightness.

In Figure 4 it can be appreciated that the main differences between the spectra in acetone of lutein (10 CDBs, one endocyclic) and β-carotene (11 CDBs, 2 endocyclic), the major carotenoids in olive oils, are the shape of their absorption bands and the location of their absorption maxima (424, 448, and 476 in the case of lutein and 454 and 480 nm in the case of the carotene). In color terms, it was seen that as a result of these differences, lutein exhibits lower values of a^* and b^* relative to β-carotene, while its value of hue was higher, which indicated that the red and yellow components in lutein were lower than in the carotene.

Although carotenoids are rather stable in their natural environment, they are very labile once extracted. As commented earlier in relation to the chlorophyll derivatives, the loss of cellular compartmentation can promote the isomerization or degradation of carotenoids, above all due to oxidation processes (Rodriguez-Amaya 1997, 1999). This fact explains why some pigments (pheophytins, mutatoxanthin, luteoxanthin) not present in the olive drupes just harvested can be found at a later stage or in the oil itself (Garrido and others 1990a, 1990b; Ranalli 1992a). In this sense, it is well known that 5,6-epoxycarotenoids (like violaxanthin, neoxanthin, and antheraxanthin; Figure 2) readily isomerizes into their 5,8-furanoid isomers in the presence of traces of acids. These isomerizations are accompanied by a loss of conjugation (Figure 5), and color changes do take place. More specifically, the isomerization of each 5,6-epoxide group is followed by a hypsochromic shift (displacement to shorter wavelengths) of the absorption maxima of about 20 nm (Eugster 1995; Rodriguez-Amaya 2001).

The typical absorption spectra in the visible region of the electromagnetic spectrum of violaxanthin, neoxanthin, and antheraxanthin and their 5,8-furanoid isomers in acetone are displayed in Figure 5. Some information regarding the color changes that these reactions cause has been reported recently (Meléndez-Martínez and others 2007). In their study it was concluded that the loss of conjugation derived from the isomerisation of antheraxanthin (10 CDBs) to mutatoxanthin (9 CDBs) was accompanied by a decrease in a^*, which, in visual terms, could be translated as a "loss in redness." The isomerization of violaxanthin and neoxanthin (9 CDBs) into luteoxanthin and neochrome (8 CDBs), respectively, resulted in a decrease in b^*, while the values of a^* did not change much. This could be interpreted as a loss of yellowness. Lastly, it was seen that the isomerisation of luteoxanthin (8 CDBs) into auroxanthin (7 CDBs) was followed by a marked decrease in b^*

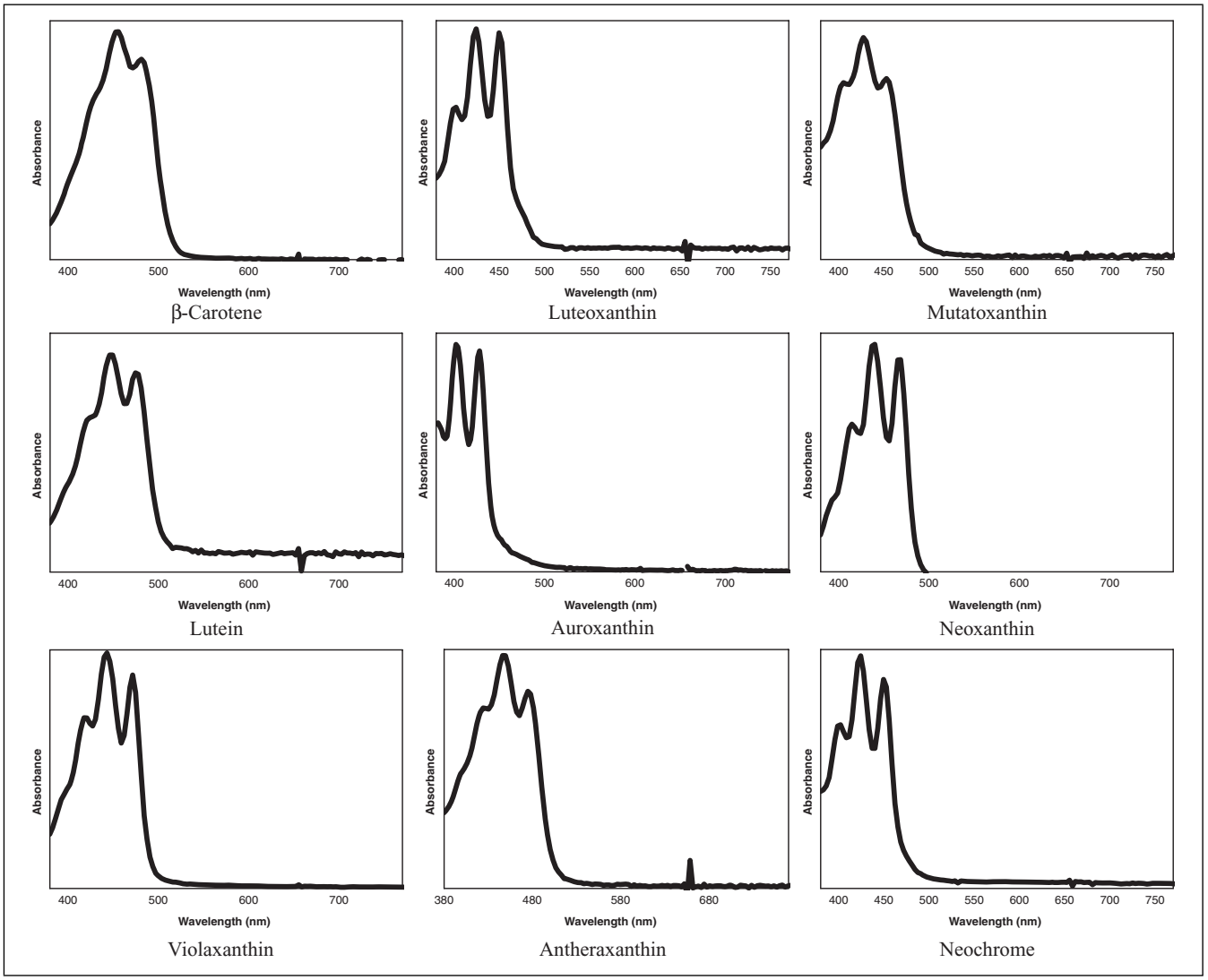


Figure 5—Absorption visible spectra (380 to 770 nm) in acetone of lutein, β-carotene, violaxanthin, luteoxanthin, auroxanthin, antheraxanthin, mutatoxanthin, neoxanthin, and neochrome.

(loss of yellowness) and a certain increase in a^*. Considering the hue, it was seen that the losses of conjugation led to increases of this parameter, which meant that the color was shifting from orange to yellowish hues (Meléndez-Martínez and others 2007).

Pigment content of olive oils

The pigment content of a wide variety of olive oils have been the subject of many studies, some of which are summarized in Table 1. High-performance liquid chromatography has been long used and remains the method of choice for the determination of olive oil pigments (Standcher and others 1987; Mínguez-Mosquera and others 1992; Psomiadou and Tsimidou 1998; Cichelli and Pertesana 2004; Hornero-Méndez and others 2005; Mateos and García-Mesa 2006). Novel approaches whose use may be interesting under certain circumstances (quality control, lack of chromatographic equipment, and so on) have also been described. Two examples are the determination of chlorophylls a and b and pheophytins a and b from fluorescence signals (Galeano Diaz and others 2003) or the rough estimation of the carotenoid and chlorophyll indexes from objective color measurements (Moyano and others 2008a, 2008b), methodologies developed with the help of appropriate multivariate statistical methods.

Olive Oil Pigments and Likely Health Benefits

Chlorophylls

The role of chlorophylls as natural pigments accounting for greenish colors and in photosynthesis is well known. However, there are some reports that hypothesize that chlorophyll pigments and related compounds may be beneficial for human health. In this sense, early in the 1980s studies indicated that some of these pigments seemed to exhibit antioxidant activity under certain conditions (Endo and others 1985a, 1985b), although the same authors also observed that they could also act as prooxidants

Table 1—Major pigments found in diverse olive oils.

Olive oil samples	Major pigments determined	Reference
Verdial variety	Pheophytin a, chlorophyll a, chlorophyll b, pheophytin b Lutein, β-carotene	(Mínguez and others 1990)
Picual, Picudo, Subbética, Hojiblanca, and Pajarero varieties	Pheophytin a, chlorophyll a, chlorophyll b, pheophytin b Lutein, β-carotene, violaxanthin, luteoxanthin, antheraxanthin, mutatoxanthin, neoxanthin	(Mínguez-Mosquera and others 1992)
Nevadillo, Hojiblanca, Picual, Marteña, Pajarero, Arbequina, and Cornicabra varieties	Pheophytin a, chlorophyll a, chlorophyll b, pheophytin b, pheophorbide a Lutein, β-carotene, violaxanthin, luteoxanthin, antheraxanthin, mutatoxanthin, neoxanthin, β-cryptoxanthin	(Gandul-Rojas and Mínguez-Mosquera 1996)
Greek olive oils	Pheophytin a, chlorophyll a, chlorophyll b, pheophytin b Lutein, β-carotene	(Psomiadou and Tsimidou 1998)
Arbequina, Blanqueta, Cornicabra, Hojiblanca, Picual, and Lechín varieties	Pheophytin a, chlorophyll a, chlorophyll b, pheophytin b Lutein, β-carotene, violaxanthin, luteoxanthin, antheraxanthin, mutatoxanthin, neoxanthin, β-cryptoxanthin	(Gandul-Rojas and others 2000)
Amfissis, Athinolia, Chondrolia, Kolovi, Koroneiki, Lianolia, Manaki, and Throumbolia cultivars	Pheophytin a Lutein, β-carotene	(Psomiadou and Tsimidou 2001)
Miscellaneous	Pheophytin a, chlorophyll a, chlorophyll b, pheophytin b Lutein, violaxanthin, neoxanthin	(Cichelli and Pertesana 2004)
Verdial, Picual, and Manzanilla varieties	Pheophytin a, chlorophyll a, chlorophyll b, pheophytin b Lutein, β-carotene	(Luaces and others 2005)
Arbequina variety	Pheophytin a, chlorophyll a, chlorophyll b, pheophytin b, pheophorbide a Lutein, β-carotene, violaxanthin, luteoxanthin, antheraxanthin, mutatoxanthin, neoxanthin, α-carotene	(Criado and others 2007b)
Arbequina and Farga cultivars	Chlorophyll a, Chlorophyll b Lutein, β-carotene, violaxanthin, antheraxanthin, neoxanthin	(Criado and others 2007)
Cerasuola, Nocellara, and Biancolilla varieties	Pheophytin a, chlorophyll a, chlorophyll b, pheophytin b, pyropheophytine a Lutein, β-carotene, violaxanthin, luteoxanthin, antheraxanthin, neoxanthin, β-cryptoxanthin	(Giuffrida and others 2007)
Arbequina	Pheophytin a, chlorophyll a, chlorophyll b, pheophytin b, pheophorbide a Lutein, β-carotene, violaxanthin, luteoxanthin, antheraxanthin, mutatoxanthin, neoxanthin, α-carotene	(Criado and others 2008)

(Endo and others 1984). Some years later, compounds related to chlorophyll a isolated from bivalves also attracted certain attention owing to their likely antioxidant actions (Yamamoto and others 1992; Watanabe and others 1993).

The interest in the possible beneficial effects of chlorophyll pigments and related compounds has re-emerged in the current decade. In this regard, some further studies that seem to indicate that they may be antioxidants have appeared (Kamat and others 2000; Lanfer-Marquez and others 2005). More importantly, some authors have reported that they may also be beneficial in the prevention of cancer (Ferruzzi and others 2002; Ferruzzi and Blakeslee 2007). Due to this renewed interest in chlorophyll pigments in connection to the health benefits they may provide, their bioaccessibility (release from the foodstuff and processing into a form that can be absorbed) and uptake by human intestinal cultured cells have been evaluated (Ferruzzi and others 2001; Gallardo-Guerrero and others 2008), since bioactive compounds must be absorbed and enter the systemic circulation to be distributed to the tissues where they exert their functions or actions.

Carotenoids

Carotenoids are much more than pigments furnishing many natural structures (petals, fruits, feathers, egg yolk, among others) with yellowish, orange, or reddish colors, this range of colors widening by association with proteins (Britton 1996). In fact, they also play key roles in photosynthesis, like for instance, the protection against deleterious photooxidative damage (Frank and Brudvig, 2004; Telfer and others 2008) and are precursors of aromas and the plant hormone abscisic acid (Lewinsohn and others 2005; Nambara and Marion-Poll 2005). Apart from these and other functions, carotenoids have attracted the attention of scientists for decades due to their nutritional importance. Thus, some of them (β-carotene, α-carotene, β-cryptoxanthin, and so on) are precursors of vitamin A. Over and above the role of some carotenoids as provitamins, a large body of evidence exists indicating that they may be effective antioxidants (Burton 1989; Olson 1993; Krinsky 2001) and beneficial in relation to the prevention or amelioration of serious human ailments like skin (Mathews-Roth 1979, 1990) and eye disorders (Snodderly 1995;

Bernstein 2002), cancer (Krinsky 1989; Wang and Russell 2000; Rock 2002; Palozza 2004), cardiovascular disease (Voutilainen and others 2006; Wang and others 2008). Although epidemiological studies indicate that the consumption of carotenoid-rich products should be encouraged (Southon 2000; O'Neill and others 2001) and many advances in the understanding of the mechanisms by which these compounds exert their beneficial effects have been made in the last few years (Mortensen and others 2001; Krinsky and Yeum 2003; Elliott 2005; Krinsky and Johnson 2005; Carail and Caris-Veyrat 2006; Kim and others 2006; Linnewiel and others 2009; Liu and others 2009), there is still some controversy about the effects of carotenoids *in vivo*. Thus, some authors have reported that they may also be prooxidant under certain conditions (Palozza 1998; Young and Lowe 2001; Lowe and others 2003; Palozza and others 2003) and even procarcinogens (Wang and Russell 1999; Paolini and others 2003).

As far as the major carotenoids in olive oil are concerned, both β-carotene and lutein are thought to provide several health benefits. The vitamin A activity of β-carotene has long been known and is not further discussed in this review. However, the interest in this compound was revived some 25 y ago in relation to its likely antioxidant activity, a line of research that is still attracting the interest of researchers (Burton and Ingold 1984; Jialal and others 1991; Tsuchiya and others 1994; Nakagawa and others 1996; Paiva and others 1999; Kim and others 2007). Likewise, much attention is being paid to its probable beneficial effects in relation to the prevention of cancer (Russell 2002; Kim and others 2007; Liu and others 2009). However, as mentioned before for carotenoids in general, there is still certain controversy about some of the goodness of β-carotene (Gaziano and others 1995; Wang and Russell 1999; Palozza and others 2003; Paolini and others 2003), in many cases derived from the design of the assays.

Lutein is also being paid much attention in the last few years in relation to its nutritional relevance (Granado and others 2003; Calvo 2005), as a result of which it is being added to both animal feeds and human dietary supplements (Breithaupt and Schlatterer 2005; Breithaupt 2007). More specifically, this pigment is being extensively studied in relation to eye health, since it accumulates along with zeaxanthin in the macula lutea of the fovea and they both are thought to be beneficial for the prevention of cataracts and age-related macular degeneration (Johnson and others 2000; Trumbo and Ellwood 2006; Schalch and others 2007; Cho and others 2008; Carpentier and others 2009). Furthermore, in some studies it is concluded that lutein may exhibit antioxidant activity (Haila and others 1996; Stahl and others 1998; Broniowska and others 2007), although it must be considered that controversy about the *in vivo* antioxidant role of carotenoids in general remains, as mentioned earlier. Two recent works also indicate that this pigment may protect against DNA damage (Santocono and others 2006, 2007). As a result of this increasing interest in lutein in relation to human health, its status (Cardinault and others 2003), bioaccessibility (Granado-Lorencio and others 2009), and bioavailability (Olmedilla and others 1997, 2002; Granado and others 1998; Lienau and others 2003) have been evaluated thoroughly in recent years.

Olive oil and pigment bioavailability

In relation to the health benefits of carotenoids, in general, it is important to bear in mind that they must be absorbed and delivered to the relevant tissues so they can exert their biological functions or actions. Being highly lipophilic, the absorption of these compounds is complex and requires their release from the food and their incorporation into micelles and subsequent uptake for the enterocytes and release to the systemic circulation. These processes are dependent on several factors, like the interaction of carotenoids with other food components and between them, the food matrix, the intrinsic characteristics of the individual, and more (Castenmiller and West 1997; Yeun and Russell 2002; Faulks and Southon 2005). For instance, it is well known that the commercial food processing and culinary practices cooking can improve the bioavailability of carotenoids (Rock and others 1998; Edwards and others 2006; Hornero-Mendez and Minguez-Mosquera 2007; Veda and others 2008; Thakkar and others 2009), so it is sensible to assume that lutein and β-carotene are more available from olive oils than from the drupes, since in the latter case, they have to be 1st released from the cells. Moreover, it must be considered that the absorption of these compounds can be improved by the simultaneous presence of oil, which means that olive oil can be a perfect vehicle for the absorption of native carotenoids and those present in other foods consumed at the same time (Fielding and others 2005; Ahuja and others 2006; Lakshminarayana and others 2007).

Legal Aspects Related to the Color of Olive Oil

Despite these examples illustrating the importance of color as a quality attribute in olive oils, when the quality parameters of these products were established, not much attention was paid to color. Indeed, the applicable legislation is far from specific in relation to the color of olive oils.

When the quality parameters of olive oils started to be defined by the competent authorities, color was left out, such that it became linked to their commercial characterization or the agreement between the seller and the buyer. In the 1st regulation of the Intl. Olive Council (IOC), where the quality requirements for virgin olive oils were established (COI 1987), the sensory attributes more appealing to the consumer were not appropriately defined. In fact, it turned out unfeasible to perform an objective classification based on them since reference methods to be used for the assessment of aroma, taste, and color were not even described (Gutiérrez 1987). This regulation at least specifies the color (subjectively defined with terms as bright, yellow to green, and so on) that some categories should have. However, the specifications were so ambiguous and subjective that the differentiation between some of them was very difficult at best. However, in later regulations this specification regarding the color of the categories disappears, to the extent that in the current regulation there is not any either (COI 1996).

During the 60th meeting of the IOC, the need of establishing an objective methodology to define the color of olive oils was raised. In this regard, a proposal of the assessment of color according to a continuous scale was set out, although the study was eventually postponed (COI 1990). In the case of the regulation of the European Union (EU) applicable to olive oils with special emphasis on analytical methods, there is not any specification relative to their color definition (CEE 1991).

The sensory evaluation proposed both in the IOC and in the EU regulations is an objective assessment of the quality of virgin olive oils that does not introduce preference or acceptability criteria. Pertaining to this, it is important to consider that the acceptability studies constitute another key aspect of the quality of the product. These studies involve the realization of appropriate sensory tests to be carried out by a large number of untrained people to determine new potential markets or set the specific attributes of olive oils that are of special interests to the consumers (Dobarganes 1994).

Anyhow, several studies on the objective specification of this attribute have come out in the last few years, it is important to

stress the need to define unambiguously the different attributes related to color (lightness, hue, chroma, for example) (Escolar and others 1994, 2007; Moyano and others 2001, 2008a, 2008b; Melgosa and others 2004, 2005).

Methodologies for the Assessment of Olive Oil Color

According to the reasons set out in the introduction, the need of having appropriate methods to define olive oil color is unquestionable; hence, this parameter has been assessed in different ways for decades. In principle, the approaches followed to this end can be readily grouped into 2 main categories, visual and instrumental methods. The visual analysis forms part of the sensory analysis of the product and can be carried out rather easily without the need of any instrumentation. Although there may be a certain vocabulary to define the colors so studied, the description is largely subjective. In contrast, to carry out instrumental measurements, a certain investment has to be made, the prices of the instruments suitable for the measurement of color ranging considerably. By means of these measurements, an objective definition of color can be attained once the reference conditions necessary to carry out the readings are set.

Visual methods

Visual analysis of olive oil and food colors in general are commonly carried out. In this regard, large panels of untrained panelists are normally considered to determine preferences, whereas trained panelists are used for descriptive assessments. Indeed, trained judges are used for quantitative descriptive analysis (QDA), which is useful for generating color descriptive terms, which can be correlated with instrumental or other type of data at a later stage.

The simplest way of assessing the color of an olive oil visually is to compare it with color scales. These scales consist of a series of colored solutions, which are rather stable and can be made from easily available colorants. Furthermore, the scale of reference solutions must encompass all the possible colors that can be found in the type of olive oils studied. It is very important to place the samples to be assessed in appropriate cuvettes, and that these match those used to contain the reference solutions, since both (the sample and the references) are eventually compared using a standardized blank of diffuse light (Naudet and Sambuc 1955; Belbin 1993).

In the case of olive oil, the bromothymol blue method (BTM) is widely used. This method is based on the establishment of a scale of indexes that do not contain reddish hues for the definition of the color of oils from olives and seeds. That is, the scale has to encompass hues ranging from yellow to green (AENOR 1963). A modification of this method for the visual color definition of virgin olive oils has also been proposed (Gutiérrez and Gutiérrez 1986).

Colored glasses can also be used as reference standards as an alternative to colored solutions. These types of glasses are used in the Lovibond method (AOCS 1992). This method consists in the visual color matching of the light transmitted through a specific surface of oil with the color of the light originated by the same source transmitted through the colored glass standards. The apparatus enables to observe the oil under controlled conditions, since the illumination is standard and the vision angle is set. The optic system is designed to help the observer see simultaneously the field of the reference blank and that of the olive oil sample. The series of reference colored glasses is numbered and comprises colors ranging from unsaturated water-white to completely saturated red, yellow, and blue colors. These colors constitute the so-called Lovibond color scale. The color matching between the samples and the references is expressed in terms of Lovibond units of red, yellow, and/or blue (Belbin 1993).

Other color scales used to define the color of oils are the Gardner scale (mainly used to classify natural and synthetic oils, lecithins, fatty acids, and some oil derivatives) (AOCS 1964), the Wesson method (applicable to all the normal fats and oils) (AOCS 1992), or the fatty acid committee (FAC) scale, used to classify nonedible grease and dark oils (AOCS 1943).

The determination of the color of olive oils presents other drawbacks, such as the obtaining of appropriate reference material itself, the fact that these are not rigorously standardized, and the possibility that they undergo color changes due to oxidation processes catalyzed by heat, light, and oxygen as time goes by. Besides, the color matching by these means is rather imprecise and is very dependent on the observer. It is commonplace that different observers match the same sample differently and that the same observer matches the same sample differently when the test is repeated (Presnell 1949; Naudet and Sambuc 1955; Pohle and Tierneh 1957). On the other hand, in relation to the impact of color in the sensory analysis of olive oils, in general, a recent study concludes that the objective intended when using blue glasses for the oil tasting, which is to conceal the color so it does not influence the sensory assessment of aroma and taste, is not fulfilled. In this sense, it was reported that such glasses fail to effectively conceal the color such that the panelist can appreciate it visually. From this it can also be inferred that the color of olive oils is paid little importance in relation to the sensory analysis to the extent that it is regarded as an "annoying attribute." This contrasts with the sensory analysis of other products for human consumption. For instance, in the case of wines, color is an important part of the sensory analysis and the panelists are expected to be sufficiently trained so as not to let themselves be influenced by it. In this study, it is concluded that the color of olive oils should also be carried out in a transparent glass so the sensory analysis is complete. In this sense, it is pointed out that the color intensity and the hue are much related to the degree of ripeness of the drupes. Additionally, the fact that unfiltered oils are acquiring certain relevance gives support to this recommendation, inasmuch as the consumer can regard olive oils having natural turbidity as more natural. On the other hand, the authors state that in order to effectively conceal the color of the oils, the chromatic characteristics of the tinted glass used for the sensory analysis should be established more carefully (Melgosa and others 2009).

To conclude this section, it can be stated that the need to reduce the subjectivity linked to the visual assessments led to the progressive development of instrumental methods. These methods also offer a series of advantages (simplicity, nondestructiveness, affordable and versatile equipment, portability, ultra-rapid measurements, possibility of automation, and so on) that can be harnessed for the quality control of foods in the field by the industry, or even in the marketplace.

Instrumental methods

Spectrophotometric methods. The Color Committee of the American Oil Chemists' Soc. (AOCS) (Agee 1948) issued a report stating that, although the system had been useful and necessary, the use of the visual colorimeters used for the Lovibond glasses should be discontinued. This measure was adopted on the basis of the advances in scientific instrumentation, such that the use of photoelectric colorimeters was advised in order to obtain a more accurate classification of the oils. This report had also as an objective to present some data on the color of olive oils obtained with a spectrophotometer and suggested a method according to which the spectrophotometric data could be used to replace the measurements made according to the Lovibond system. In this

regard, the results obtained after measuring 9 oil samples in a spectrophotometer (at 550 nm and using CCl_4) and according to the Lovibond system in 2 laboratories were compared. The outcomes indicated clearly that the red Lovibond colors could be easily estimated, except in the case of the green oils, for which low Lovibond values were obtained. Additionally, it appeared that it did not matter that the oil was diluted or not in CCl_4.

In a later study under the auspices of the Color Committee of the AOCS, more than 30,000 spectrophotometric, Lovibond, and other visual assessments of the color of different oils (soy, cotton, and peanut) were made in 9 laboratories. After statistically analyzing the data in 4 of the laboratories, 4 equations were proposed. By recommendation of the Color Committee of Oils, one of these equations was unanimously accepted by the AOCS (Agee 1950). In this equation, which appears in the current spectrophotometric method for the determination of the color of soy, cotton, and peanut oils (AOCS 1989a, 1989b), the transmittance readings at 460, 550, 620, and 670 nm are taken into account, with their corresponding coefficients:

$$R = 1.29\ D_{460} + 69.7\ D_{550} + 41.2\ D_{620} - 56.4\ D_{670}$$

where

R = red Lovibond
D = optical density

The correlation found with the red Lovibond color was very high ($r = 0.993$). However, this equation presents some inconveniences. The most serious of them is related, evidently, to the presence of a negative factor in the equation, because, when the oils are green, this is so considerable that a negative red Lovibond is obtained. Put otherwise, the oils would have an unreal color (Naudet and Sambuc 1955; Pohle and Tierneh 1957).

The spectrophotometric measurement has been proposed to establish a method to control de decoloration of oils, considering especially the olive oils in which absorption maxima corresponding to the pigments appear. The absorption spectrum changes considerably as a function of the relative percentages of pigments occurring in them (Stella 1966). In the case of olive oil, the visible spectrum presents 7 regions of maximum absorption: 410 to 420 nm, 450 to 455 nm, 480 to 485 nm, 530 to 535 nm, 560 to 565 nm, 610 to 615 nm, and 660 to 673 nm (Figure 6). Taking these facts into consideration it is important to bear in mind, among other circumstances, the likely interaction between colored compounds with similar absorption maxima, the influence of the conditions of the medium (acidity, temperature, refractive index) or transformations of the original pigments into derivatives with other chromatic characteristics. For the spectrophotometric measurements, the extinction coefficients corresponding to the 7 regions are calculated and by averaging them the mean extinction coefficient of the oil is obtained (K_0). From K_0 a decoloration index (I_d) is proposed, which is calculated by the following equation:

$$I_d = K_0 - K_d/K_0$$

where K_d corresponds to the mean extinction coefficient of the decolored oil, such that I_d ranges between 0 and 1.

In a more practical way, the 7 wavelength regions can be grouped into 3 (410 to 480, 535 to 565, and 610 to 660 nm, which correspond to yellow, purple–violet and green–blue colors, respectively) and the partial index of each can be calculated.

In relation to the pigments, formulas for the calculation of carotenoid and chlorophyll indexes (CI and CLI, respectively) based on spectrophotometric readings have been proposed (Papasseit 1986):

$$CI = A_{500} \times 100$$

$$CLI = A_{670} - (A_{630} + A_{710}/2) \times 100$$

However, all the efforts made to find a suitable spectrophotometric method for the meaningful color definition of olive oils were based on a rather inappropriate premise, that each new method must provide values that can be translated into Lovibond colors. The outcome was that, after many years of work, assembling numerous data, and testing several instruments, an acceptable methodology has not been achieved. However, the insistence in relating the colors of oils with the values obtained according to the Lovibond method was, to some extent, justified due to the services given to the industry and the market for many years. The customary use of this methodology precluded the rapid transition to others. Additionally, some people are so used to the Lovibond method that the values obtained from it represent to them the visual sensation of the color far better than other color parameters. In this regard, it is possible that this has also contributed to the fact that the application of spectrophotometry to the color definition of oils in general has not been approached correctly. Nonetheless, in the particular case of olive oil, this problem still does not exist, because there is not yet a standard methodology for its color assessment. This fact, that on many occasions could be a serious drawback due to the lack of reference methodology to be used as foundation, can also be an advantage.

On the other hand, considering that unfiltered oils are growing in importance it is also important to determine if the color of these oils should be inferred from absorbance measurements or by other means, as the high turbidity of these samples could be a problem.

The application of tristimulus colorimetry to olive oils

The physiological sensation to examine the appearance of a transparent liquid is dependent on 3 factors: the amount of radiant energy emitted by the source at each wavelength (emission spectrum of the source), the way in which this energy is transmitted by the sample observed (emission spectrum), and the response of the eye of the observer to the radiations of different frequencies (curve of sensitivity of the eye). The 2nd factor is the most important on which to base the proposal of the appreciation of the oil color. In this sense, what it is sought is to define a color from the sensations perceived by the human eye, copying in a "natural way" the language of the colors and the paintings. Thus, a color is characterized by its hue (yellowish, orange, greenish), its purity (the basal hue is more or less mixed with the white) and its brightness (the color is more dark or more bright).

The tristimulus colorimetry deals with the chromatic specification of color abiding by the basis of the trichromatic theory. The interest in the application of this theory to the color definition of oils started decades ago, above all, after it was demonstrated that their color assessment by visual comparisons or empirical equations yielded few reliable outcomes. Due to the variable amounts of the different pigments present in the oils, it is not possible to select only one wavelength at which the transmission is an exact function of the total or visual transmission of the oil. In this regard, it is considered that the expression of the results in terms of tristimulus values must be a more satisfactory method (Presnell 1949; Naudet and Sambuc 1955; Bigoni 1963).

The problem of the choice of the most adequate solvent to be used as blank reference has been tackled, as well as the validation

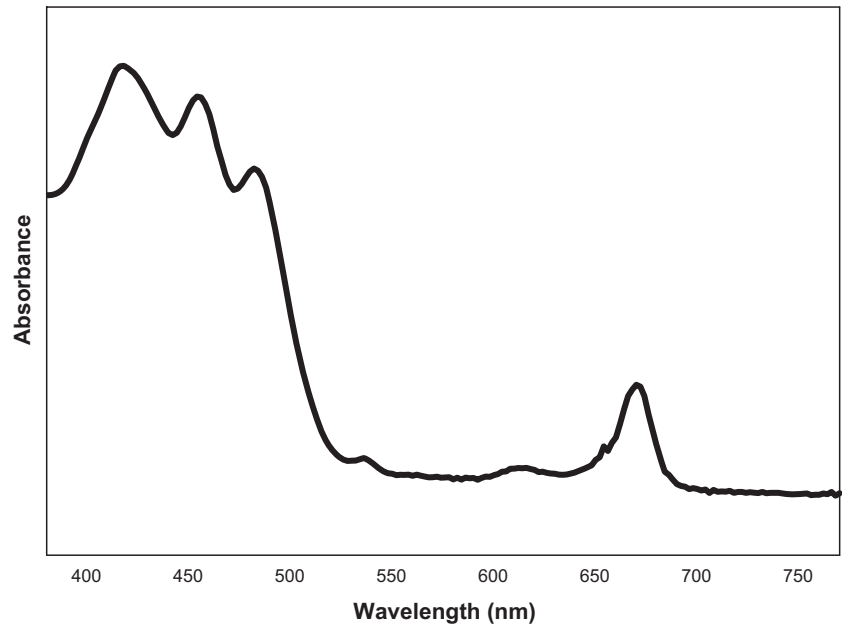


Figure 6—Typical visible absorption spectrum of an olive oil.

of the Lambert–Beer Law and its application to fat solutions in different solvents and at different sample thicknesses. Additionally, the trichromatic theory has been applied in different cases like raw fatty compounds, decoloration of fats, and refined fats (Naudet and others 1956).

In the particular case of olive oils, the transmittance curves of a series of representative samples (3 refined oils, 3 blends of virgin and refined oils, 4 commercial olive oils, and 12 virgin olive oils obtained by the systems of pressing and partial extraction in the experimental olive mill of the Instituto de la Grasa (Sevilla, Spain) were obtained. From these samples, their coordinates in the International Commission on Illumination (CIE, from its name in French: Commission Internationale de l'Eclairage) system were calculated as a starting point to deduce the methodology to be applied to assess the color of those oils (Castro and others 1955). More specifically, the chromaticity coordinates (x,y) and the factor of luminance (Y) were calculated using the illuminant C and the tristimulus values X,Y,Z. In the chromatic diagram, it was observed that it was possible to trace a line that, with a very good approximation, represents the location of the chromaticity of olive oils. This fact would enable by establishing a scale along that line, to reduce the specification of the chromaticity of olive oils to only one parameter, and that of the "complete color" to 2 parameters, by including the luminance, which is not included in the diagram. On the other hand, the fact that this line was largely straight made it evident that it would be easy to design a simple colorimeter to determine the parameter to be used for the color definition without having to resort to standards. In relation to this, several simplified methods have been proposed in order to reduce the numerous calculations necessary to work out the trichromatic coordinates (above all when computing was not as advanced as it is nowadays) or to enable its application in the case that the necessary instrumentation to register the complete spectrum was unavailable. Likewise, the use of simple colorimeters to register only several wavelengths has also been proposed. In this regard, it seemed feasible to obtain the tristimulus values with a good approximation from functions of the transmission values at certain correctly chosen wavelengths. The procedure consisted basically of the application of Hardy's method of selected coordinates (Hardy 1936), although reducing the number of coordinates to a great extent. Presnell (1949) performed it from oils clarified by filtration and dehydration if necessary. For this purpose, 5-mm cuvettes were used, except for very dark oils, for which narrower cuvettes were employed and the results were finally corrected to 5-mm pathlengths. As blank reference, both water and CCl_4 could be used (although the latter was recommended because its refractive index is closer to that of most of the oils), while the illuminant C was considered as reference for being the most widely used at that time. For the original calculation of the tristimulus values, oils from different origins exhibiting varied transmission spectra were used and 30 coordinates were selected. Subsequently, 3 coordinates were selected and the corresponding factors that could be applied to achieve a good approximation to the tristimulus values previously calculated were found. Eventually, the following expressions for X,Y,Z., obtained from the transmittance readings at 445, 555, and 600 nm, were arrived at:

$$X = 0.2\,T_{445} + 0.15\,T_{555} + 0.65\,T_{600}$$

$$Y = 0.1\,T_{445} + 0.7\,T_{555} + 0.2\,T_{600}$$

$$Z = 1.2\,T_{445} + 0.06\,T_{555}$$

Although the approximate values obtained applying these equations were in accordance with the values obtained by the method of the selected coordinates, Sambuc and Naudet (1956) made some observations to these results:

- If the equations proposed by Presnell are applied to a perfectly transparent solution ($T(\lambda) = 100$), it can be observed that the trichromatic coordinates do not coincide with those of the point C (colorless).

- X and Z are erroneous and the scattering of the results is considerable, especially in the case of Y.
- The samples surveyed by Presnell were very bright oils with little variation in brightness.

The authors tried to enhance the results increasing to 4 the number of degrees of transmission used and choosing conveniently the wavelengths (444.4, 495.2, 551.8, and 624.2 nm), such that the following equations were eventually obtained:

$$X = 0.19\,T_{444.4} + 0.33\,T_{551.8} + 0.46\,T_{624.2}$$

$$Y = 0.17\,T_{495.2} + 0.63\,T_{551.8} + 0.20\,T_{624.2}$$

$$Z = 0.94\,T_{444.4} + 0.24\,T_{495.2}$$

As a result of these modifications, the deviation observed between the reference values and the practical values were markedly reduced, considering that the main source of error was due to raw and little refined oils that exhibited a marked variation of transparency in a very narrow region of the spectrum.

According to Bigoni (1963) the previous methods were based in the relative continuity of the absorption spectrum of the oils, normally without any characteristic band, which justifies partially the selection of only 4 wavelengths in correspondence to the 3 primary sources. Thus, some inconveniences were pointed out:

- The longest wavelength for the expression of the tristimulus values was 625 nm, so the absorption band due to chlorophylls at 670 nm, which is characteristic of some oils like olive oils, was not considered.
- The method restricts itself to give as results of the measurements the values XYZ. Such numerical values are not appropriate to convey an exact idea of the color, with the exception of the brightness (Y).

Consequently, the author developed a modification of the method, based on the following principles: to add to the equation of X (that considers the region 600 to 700 nm) another term that took into account the absorption band due to chlorophylls and the proposal of 2 simple equations that manage to express directly the values of saturation (S) and hue (λ), avoiding the graphical representation:

$$X = T_{660}/0.05 + T_{625}/0.40 + T_{550}/0.37 + T_{445}/0.19$$

$$Y = T_{625}/0.19 + T_{550}/0.14 + T_{495}/0.17$$

$$Z = T_{495}/0.18 + T_{445}/0.82$$

$$S = 100(1 - 3Z/(X + Y + Z))$$

$$\lambda = 872 - 173.5\,\log(100(Y - Z)/(T - 3Z))$$

More recently, a methodology for the rapid determination of the color of virgin olive oils from the absorbance or transmittance readings at 480 and 670 nm of the pure samples has been proposed (Escolar and others 1997). These values correspond to the absorption maxima of the major pigments accounting for the color of the samples and, therefore, for the main absorption bands in their visible spectra. Indeed, the visible spectrum of a virgin olive oil can be simulated by means of an adequate combination of the spectra of carotenoids and chlorophylls. Moreover, simplified methods for the determination of the color of olive oils based on the application of the analysis of characteristic vectors have been proposed (Ayala and others 1994; Moyano and others 2001).

The use of filter photocolorimeters to assess the color of olive oils has also been described. When the filters are illuminated by means of a well-defined source and the emerging light is received on a photoelectric cell, the response curve of the cell as a function of the wavelength is identical to the contribution of each of the primary in that source. In other words, the light of each filter acts like a primary source. One of the most widely used filter photocolorimeters is the so-called Hunter photocolorimeter. With this apparatus the X,Y,Z values of the samples can be calculated from the degrees of transmission R,G,B read with each filter. Another similar apparatus has been devised from some of the simplified methods to determine the trichromatic coordinates (Cruz and others 1956; Sambuc and Naudet 1956, 1960; Bigoni 1963).

Up to this point, we have dealt with the development of instrumental methodologies that can be used to overcome the subjectivity linked to visual measurements. However, the comparison between different methods for the color assessment of oils has also been the objective of several studies. For instance, the correlation existing between different instrumental and visual methods to measure the color of rapeseed oils has been determined (Brát and others 1988, 1993).

In recent years, the BTM has been the subject of several investigations, in which some of the limitations of this methodology were pointed out. Thus, the trichromatic coordinates corresponding to 90 virgin olive oil samples and to these standard solutions were evaluated to examine the validity of the BTM scale. Interestingly, it was observed that the scale only matched part of the oils studied. Furthermore, considering the typical spectra of one oil and that of a standard solution, it was suggested that there may be problems of metamerism (the phenomenon by which 2 stimuli with different spectral composition are visually perceived as equal) that could invalidate even more the use of the BTM scale in the industry. As a result of these observations, it was concluded that certain corrections were necessary to take advantage of the well-known practical utility of this scale (Burón and others 1989). Moreover, it has been demonstrated that there is a clear chromatic degradation of the standards with time, which advised against the use of this system (Moyano and others 1999; Melgosa and others 2001). In another investigation, conclusions concerning the low precision, accuracy, and uniformity of the scale were drawn and additional suggestions for its enhancement were provided (Melgosa and others 2000).

In a comparative study, several types of oils subjected to thermal oxidation were used to calculate the chromatic parameters from the standard methods recommended by the CIE and several simplified methods. As a result, it was concluded that the latter methods could only be applied to certain chromatic parameters and certain oils and that, therefore the standard methods recommended by the CIE must be applied for a complete study of the color parameters of the samples (Guillén and others 1991).

In another interesting study, the mistakes made in the calculation of the tristimulus values of olive oil by methods based on the use of several selected coordinates and an increase in the number of such coordinates were analyzed. For this purpose, 10 methods were evaluated. Four were old methods developed for other kinds of oils but eventually used in olive oils. The other 6 were methods that used a large number of transmittance values and

that were developed to determine the influence of the number of data considered to calculate the tristimulus values. The study demonstrated, as expected, that a large number of coordinates provide better results in the definition of olive oil color (Escolar and others 1994).

In recent years, new studies concerning the objective measurement of olive oil color have been published. In one of them, a uniform color scale for virgin olive oils was developed from the chromatic parameters corresponding to several hundreds of Spanish olive oils from different varieties and origins. More specifically, this scale proposed a new set of color standards that appear more appropriate than those proposed by the BTM (Melgosa and others 2004). Furthermore, the CIE 1976 (CIELAB) color space for virgin olive oil has been recently determined and used to classify the color of over 100 Spanish samples of diverse origin (Escolar and others 2007).

To conclude, it is important to mention that some of these methods are being widely used for several purposes, like characterization (Mincione and others 1996; Motilva and others 1998; Criado and others 2008) or assessment of color changes during processing (Ranalli 1992a; Ranalli and Angerosa 1996) or oxidation tests (Ranalli and Angerosa 1996; Ceballos and others 2003). Interestingly, the color of olive oils has also been related to their pigment content (Mínguez-Mosquera and others 1991; Moyano and others 2008a, 2008b), a research field that is susceptible to expand in coming years due to the growing interest in these compounds owed to their likely health benefits.

Concluding Remarks

Olive oil is a key component of the praised Mediterranean diet, which keeps on attracting the interest of scientist due to the health benefits associated with its consumption. Its color is of great importance in relation to the acceptability by the consumers, but also from an industrial point of view. In recent years, the interest in the pigments accounting for this attribute has revived due to accumulating evidence indicating that they may be beneficial for the prevention or alleviation of serious human disorders directly related or not to oxidative stress (certain types of cancer, cataracts, age-related macular degeneration, and more). Although the legislation is very lax in relation to the color assessment of olive oils, according to the facts mentioned above, there is no doubt that it is necessary to design meaningful standardized methods to define this attribute. In this sense, some studies conducted in the last few years indicate that the widely accepted visual assessments of olive oils present serious problems that should be overcome by using objective instrumental methods. These can be used not only to define the color of the products, but also to estimate the contents of chlorophylls and carotenoids, which is very interesting and can be applied in quality control and other situations.

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