Factors that influence color degradation in extra virgin olive oils

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Nowadays olive oil is considered a global consumption product recognized by the numerous benefits it confers to human health. Its quality is regulated by the International Olive Council that establishes parameters concerning free acidity, peroxide index and organoleptic characteristics based on sensations perceived by the senses. Color is one of the most immediate organoleptic properties of olive oils determinant in consumer's choice. As olive oil is a natural product, pigments are affected and partially destroyed by the oxidative and degradation processes that the oils suffer over time during the conservation prior to consumption. This alteration of is therefore perceived today as one of the most important changes affecting the organoleptic quality of the product. The objective of this chapter is to contrast the color changes experienced by different virgin olive oils subjected to standard storage conditions and evaluate the relationship between the color alteration and the alteration of its chemical composition.

Keywords: Extra Virgin Olive Oil; Storage; Color degradation; Chemical Composition

1. Intoduction

Extra virgin olive oil (EVOO) 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 [1]. This is due to its complex chemical composition which comprises various antioxidant substances that can be both nutritional and therapeutic. Polyphenols, sterols and fatty acids are the most influential constituents in terms of health benefits that can be found in considerable quantities in olive oil and that play a major role in human metabolism being simultaneously influent in factors such as stability, flavour and color of the oil [2].

Olive oil currently represents a small share of the whole vegetable oil market but its use is gaining ground, increasing worldwide, thanks not only to its potential health benefits but also to its unique sensory and nutritive qualities, since it is the only oil that is consumed by direct obtaining. Consumers are increasingly demanding least-processed high quality foods, and requiring quality to be maintained during a long period between purchase and consumption. Food must not only remain safe but also minimise the unwanted changes produced during the storage in the sensory quality, so it is important to evaluate the variations in a natural product as EVOO is.

2. Quality criteria in virgin olive oils

2.1 Chemical analysis

According to the International Olive Council (IOC) [3] virgin olive oils are the oils obtained from the fruit of the olive tree (*Olea europaea* L.) solely by mechanical conditions, paying particular attention to the thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration. Olive oils fit for consumption as they are include:

Extra virgin olive oil: virgin olive oil which has a free acidity, expressed as oleic acid, of not more than 0.8 grams per 100 grams, and the other characteristics of which correspond to those fixed for this category in the IOC standard.

Virgin olive oil (VOO): virgin olive oil which has a free acidity, expressed as oleic acid, of not more than 2 grams per 100 grams and the other characteristics of which correspond to those fixed for this category in the IOC standard.

Olive oil: olive oil which has a free acidity, expressed as oleic acid, of not more than 3.3 grams per 100 grams and the other characteristics of which correspond to those fixed for this category in the IOC standard. This designation may only be sold direct to the consumer if permitted in the country of retail sale. If not permitted, the designation of this product has to comply with the legal provisions of the country concerned.

The EU regulation [4] establishes upper limit values for different oxidation indexes which are shown on Table 1.

Table 1 Particular characteristics of olive oils according to ICO trade standard 2015 [3] and Commission Regulation 1348/2013 [4].

	European Commission Regulation /International Olive Council trade standards		
Parameter	**Extra virgin olive oil**	**Virgin olive oil**	**Olive oil**
Free acidity (as oleic acid)	≤ 0.8 g % w/w	≤ 2.0 g % w/w	≤ 3.3 g % w/w
Peroxide value	≤ 20 meq O_2/kg oil	≤ 20 meq O_2/kg oil	≤ 20 meq O_2/kg oil
K_{232}	≤ 2.50	≤ 2.60	-
K_{270}	≤ 0.22	≤ 0.25	$\leq 0.30^{+i}$
ΔK	≤ 0.01	≤ 0.01	≤ 0.01

The regulation also establishes other chemical parameters related to the quality criteria of the EVOO such as the content of volatile matter or impurities and the content of phenols not detailed in this chapter.

2.2 Sensory analysis: the relevance of color

Color and appearance constitute the first contact we have with food determining our preferences and influencing our choices. Color is one of the first sensory attributes of food valued by consumers, especially in EVOO, and it can be considered a quality parameter that highly influences its acceptance and preference [5].

EVOO is obtained by simple pressing of olive fruit (Olea *Europaea*), as a natural product, it has its own color ranking from dark green to pale yellow depending on the olive variety [6, 7], the agricultural practices such as irrigation [8], the maturation index on ripeness [9] or the oil extraction process [10]. Olive fruit color suffers changes as it grows, ripens and matures moving from deep green to yellow- green and then to purple and black. The green color of the tissue is provoked by chlorophyllic and carotenoid pigments, and their concentration decreases progressively during ripening that gives way to the synthesis of anthocyanins which first appears as spots on the skin, covering later more and more of the fruit surface dyeing it in purple until full maturation, where it becomes black. Anthocyanin synthesis produces finally the pigmentation of the whole fruit invading the interior of the pulp. Only chlorophylls and carotenoids, which are fat soluble, are transferred to the EVOO, giving its highly valued natural color [15].

The extraction process of olive oil entails, to a lesser or greater extent according to the extraction conditions, a loss of pigments, which in terms of total pigment content, affects the chlorophyll pigments more than the carotenoids. Although chlorophylls and carotenoids in food are mainly valued for its chromatic function, recent research has demonstrated that these compounds can be considered as quality indicators for the end product. The pigments detected in EVOO can be divided in the ones coming from the fresh fruit as chlorophylls a and b, lutein, β-carotene, violaxanthin, neoxanthin, antheraxanthin, and β-cryptoxanthin, and those formed during the extraction process, pheophytins a and b, luteoxanthin, auroxanthin, neochrome, and mutatoxanthin. The formation of these pigments is due to the fact that during the milling process a series of acids is released from the tissues of the fruit, which in the malaxation and centrifugation period favors the pheophytinization of chlorophylls and the isomerization of the carotenoids [16].

Many factors, both agronomic and technological can affect the EVOO pigment profile to the point that color has been proposed by some authors as a characterizing factor used as quality index related to olive variety and oil extraction method [16, 17].

The objective measurement of color is of great importance for food producers due to the relationships between such attribute and the acceptability of food by consumers [14]. Despite its importance as a sensitive attribute, no objective standardized method for measuring EVOO color has yet been established. The most accepted system in worldwide industries seems to be the CIELAB colorimetric system, its application to EVOO samples provides better results than those obtained by visual methods [15].

3. Factors that influence the alteration of extra virgin olive oil quality during storage

The assurance of stability and quality of food products is therefore a matter of great importance for industrial producers. EVOO has generally a relatively long shelf-life, regulation considers periods between 12 to 18 months as the maximum storage period from bottling to consumption during which only minor changes of sensory characteristics occurs [16]. EVOO‘s nutritive properties, taste, aroma and color distinguish it from other edible vegetable oils, hence, if the worldwide prestige of EVOO is to be sustained, its quality needs to be maintained and assured throughout its commercial shelf life. It is a matter of great concern for the olive oil industry to preserve the positive attributes of oil during the time elapsing from production to bottling, and up to purchasing and consumption.

Several accelerated oxidative stability tests have been developed for fats and oils in order to obtain analytical results in a short period of time. Although these methods are useful for determining the relative oxidative stability of products,

the main drawback of the accelerated assays is that the autoxidation process takes place under drastic conditions, quite unlike those typically occurring under normal storage conditions. In fact, there is generally a lack of correlation between the VOO stability measured by means of accelerated tests and under normal storage conditions or shelf life [17].

During storage, the quality of EVOO may deteriorate because it can undergo both biological and chemical processes due to the hydrolysis of triglycerides caused by enzymes or/ and a chemical oxidation of fatty acids, promoted by the presence of oxygen and free radicals which is the main cause of EVOO degradation, resulting in the development of unpleasant odors, flavors, and on the long term a loss of nutritional quality [18] that could reduce its commercial value.

3.1 Hydrolysis of triglycerides

Hydrolysis usually produces an alteration of oil when enzymes and water are present. Under these conditions, the aqueous phase, consisting of a small quantity (approximately 0.5 %) of vegetation water, contains enzymes and, in particular, lipase, which is able to hydrolyse triglycerides to release free fatty acids and, as a consequence, increases the acidity of the oil. That reaction is promoted by storage temperatures higher than 18–20 °C [11]. If, in mill, olive oil is not separated from the sediment as quickly as possible the small drops of the emulsified water slowly settle on the bottom of the container forming a layer of sediment that contains sugars, proteins and enzymes which can ferment producing mostly short-chain fatty acids that provoke the organoleptic defect of muddy sediment or putrid.

3.2 Oxidative rancidity

Oxidative rancidity development has been recognised as the predominant cause of oil deterioration during storage [16]. Autoxidation is reaction between unsaturated fatty acids, regardless of whether they are in their free state or esterified as a triglyceride molecule, and oxygen.

The main compositional factors of olive oils that determine their susceptibility to oxidation are the fatty acid composition and inherent antioxidant compounds. The types of fatty acids present in the oil, and in particular their number of double bonds, determine the type and extent of chemical reactions that may occur during the storage period.

The oxidative deterioration of EVOO can be delayed by employing suitable methods like maintaining cool and stable storage temperature conditions and avoiding light exposure, but oxidation cannot be avoided [19]. Furthermore, EVOO provides a rich source of natural antioxidants. Polyphenols, sterols and fatty acid are present in considerable quantities in olive oil and play a major role in human metabolism, being simultaneously influent in factors such as stability, flavour, and color of the oil [6]. These include carotenoids, tocopherols and phenolic compounds which may act, by different mechanisms, to confer an effective defence system against free radical attack. Some authors have estimated their contribution to oil stability, that of phenolic compounds being around 30%, fatty acids 27%, α-tocopherol 11% and carotenoids 6% [20]. EVOO differ from other edible oils in the abundance of oleic acid (monounsatured), a medium content of palmitic and stearic acids (satured) and a low percentage of polyunsatured fatty acids like linoleic and linolenic. Polyunsatured fatty acids are oxidised faster than monounsatured ones, this explains the higher stability of EVOO in comparison with other vegetable oils where the percentage of polyunsatured fatty acids can reach levels above 40%.

3.2.1 Influence of temperature and oxygen concentration

It is not easy to differentiate the individual effects of temperature and oxygen on the oxidation process as strong interactions exist between them [22]. A temperature higher than 20–22 °C is dangerous because it increases the risk of oil autoxidation. The speed of fatty acids oxidation in the oil depends, in fact, on the storage temperature, which must be controlled between 13–18 °C. The most dangerous risk factor for oxidation of EVOO is however contact with air. During storage, in the presence of oxygen, the oil can be oxidised because of the activity of the lipoxidase enzymes or by a chain reaction due to free radicals. Data derived from Di Giovacchino et al. [23] indicates that the risk of oxidation is greater when the oil occupies only a small part of the container, thus favouring continued solubilisation of oxygen in the oil and, therefore, reactions with fatty acid radicals and the formation of hydroperoxides.

3.2.2 Light exposure

Natural or artificial light exposure is a risk factor in EVOO stability. Light may stimulate some photosensitizers able to produce singlet oxygen which promotes a rapid polyunsaturated fatty acid oxidation.

Chlorophylls and pheophytins have a prooxidant action in the presence of light; they act as catalysts in the formation of singlet state oxygen, which can react directly with the double bonds of oleic, linoleic, or linolenic fatty acids, thereby generating reactive species of oxygen. Thus, chlorophylls and their derived pigments enhance the early phases of the process of autoxidation and generate allyl hydroperoxides. [20]

It has been demonstrated that oils stored in light displayed significantly lower tocopherol, carotenoid and chlorophyll contents than the oils kept in the dark [18]. The oils kept in the dark mainly contained products of primary oxidation, while the oils kept in the light contained products of secondary oxidation, describing light as the main cause for the increase in absorbance at 270 nm and especially for the loss of oil color. In addition, the oils kept in the light showed

significantly higher values of triglyceride oligopolymers which are considered to be good indices of the level of oxidation of edible oils and fats owing to their high stability and low volatility.

As expressed above to reduce the speed of oil oxidation, it is recommended to store the oil in a fully filled container where the volume of air must be below 3–5 % of the total volume, well closed, in the dark and at a temperature between 13 °C and 18 °C.

4. Relationship between color degradation and oxidative deterioration

Lipid oxidation has been acknowledged as the major problem affecting edible oils, as it is the origin of important deteriorative changes in their chemical, sensory, and nutritional properties [24]. As lipids oxidize, they may form hydroperoxides, which are susceptible to further oxidation or decomposition into secondary reaction products such as aldehydes, ketones, acids, and alcohols. In many cases, these compounds adversely affect flavour, aroma, taste, nutritional value, and overall quality [25].

The EVOO used for this autoxidation and color degradation study were selected between 16 spanish olive oils varities cultivated in the west Mediterranean area (Alfafarenca, Arbequina, Arbosana, Carrasqueña, Changlot, Cuquillo, Dotó, Dulce, Farga, Llumero, Menuda, Morruda, Picual, Rojal, Serrana,Villalonga) on the basis of their color degradation due to storage time. In a previous research we found that the varieties mentioned above could be classified according to their color degradation after 3 years of storage in the dark and with a room temperature varing from 18 ºC to 26ºC in 3 groups: group nº 1 encompassed the varieties Arbequina and Cuquillo, group nº 2 Changlot and Menuda, and group nº 3 the rest of oils. Varieties Cuquillo, Farga, Menuda and Serrana have been chosen in this study as a representative sample of each group given their conditions of homogeneity in the group to which they belong

The color differences between fresh oils and oxidized oils are represented in Fig. 1. Values of the chromatic ordinates L*, a* and b* of the selected oils are shown in Table 2. Luminosity values (L*) increased slightly for Cuquillo and Menuda oils while no difference was found in this parameter for Farga and Serrana samples.

The storage appear to have a significant effect on the chromatic ordinates a* and b* for all the tested oils which correspond to the green/yellow zone in CIELAB space. The more negative the value of a* coordinate the greener the color of the oil, and the more possitive the value of b* the yellower the color of the oil. Values of both a* and b* above -10 and below 10 respectively shows oils with very litlle coloration, almost transparent. As we can see in Fig. 1 and Table 2. all oils except Cuquillo presented after 3 years of dark storage values of a* and b* between -10 and 10, that's to say, all samples except Cuquillo have lost the caractheristic color that identifies EVOO. Although there is no general agreement about the visual threshold for a normal observer to appreciate the CIELAB color difference it is ussually considered that changes in oil color could become noticeables between changes of 1 and 3 units. In our study, Farga is the less colored oil with the higher value of a*combined with the lower value of b* (chroma = 5,37), followed by Serrana that reached a chroma value of 7,99, on the other side, Cuquillo presents after the storage a chroma value of 31,33.

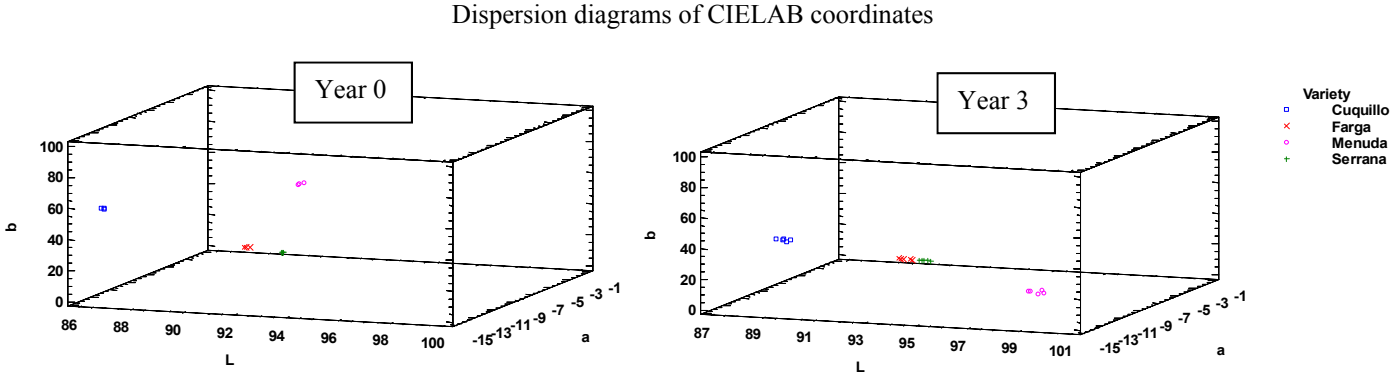

Fig. 1 Dispersion diagrams of CIELAB coordinates (L, a, b) for year 0 (oil extraction year) and year 3 (after 3 years of storage in darkness).

Table 2 CIELAB coordinates for 4 different oil varieties measured in the year of the oil extraction (L_0, a_0, b_0) and after 3 years of storage in darkness (L_1, a_1, b_1) (n = 5; mean value ± standard deviation).

Variety	L_0	a_0	b_0	L_1	a_1	b_1
Cuquillo	86,61 ± 0,09	-14,52 ± 0,07	58,79 ± 0,14	87,40 ± 0,19	-8,72 ± 0,26	30,10 ± 1,48
Farga	89,99 ± 0,12	-8,60 ± 0,03	22,18 ± 0,16	89,88 ± 0,23	-2,54 ± 0,12	4,74 ± 0,42
Menuda	94,20 ± 0,05	-14,51 ± 0,18	81,45 ± 0,11	97,31 ± 0,31	-8,89 ± 0,37	5,50 ± 0,22
Serrana	90,72 ± 0,05	-6,84 ± 0,11	15,51 ± 0,25	90,76 ± 0,17	-5,75 ± 0,02	5,56 ± 0,14

Storage has not only produced a variation of the coloration of the oils but also a change on their chemical composition as the parameters of oxidation demonstrate (Table 3).

The biologically synthesized fat is neutral, that's to say the contained oil in the healthy olive that is in the tree has 0% of free acidity. The presence of free fatty acids is, therefore, a resultant abnormality, among other factors, of the poor state of the fruits, poor treatment, or poor conservation of the same. A very low acid percentage corresponds with a high quality oil. Values close to 0.1 indicate a perfect state of the olive and a correct manipulation of the fruits during the oil extraction. The current regulation considers the limit for EVOO in 0.8º. It is, however, very frequent to find commercial oils below this limit (usually two to five tenths of acidity). As shown on Table 3, all the samples had initially very low values of free acidity expressed as percentage of oleic acid, three years after, all oil varieties except Menuda showed acidity levels below the 0,8º required by the regulation for EVOO. Menuda oil has deteriorate its quality from according to this parameter from EVOO to VOO.

Fats oxidize on contact with oxygen in the air, when a vegetable fat begins to oxidize various compounds are formed; among them are peroxides, which are considered the first oxidation products. The peroxide value determines the primary oxidation state of an oil before the smell and stale taste is appreciated. In our study, after 3 years of storage the peroxide value for the oils of Farga and Serrana varieties are far above the limit of 20 meq of O_2/kg set by de European Union and by the International Olive Oil Council (Table 3), and therefore would not meet official quality standards for EVOO.

K232 value indicates the initial oxidation process of a vegetable oil. As it occurred with the peroxide value, the samples that have shown higher K232 values are Serrana (7,24) and Farga (3,81) with values that exceed the threshold established by the regulation for virgin oils. These data go hand in hand with those obtained for the level of peroxide

K270 parameter detects a more advanced oxidative state. As the oxidative process progresses, the peroxides are modified obtaining components that absorb ultraviolet light at a different wavelength (270 nm) than the hydroperoxides. Following the criterion of K270 value, Serrana was the oil which has suffered more secondary oxidation followed by Farga and Menuda, all of three showed values above the limit indicated by the olive oil regulation after 3 years of storage. Secondary oxidation compounds are usually thought to be the most detrimental to human health [26], which makes K270 one of the most important quality parameters.

Table 3 Quality parameters for 4 different oil varieties measured in the year of the oil extraction (t_0) and after 3 years of storage in darkness (t_1) (n = 5; mean value ± standard deviation).

Variety	Free acidity t_0 (as oleic acid %w/w)	Free acidity t_1 (as oleic acid %w/w)	Peroxide value t_0 (meq O_2/kg oil)	Peroxide value t_1 (meq O_2/kg oil)	K 232 t_0	K 232 t_1	K 270 t_0	K 270 t_1
Cuquillo	0,23 ± 0,01	0,34 ± 0,01	12,63 ± 0,13	20,30 ± 0,21	1,90 ± 0,03	2,37 ± 0,12	0,12 ± 0,01	0,17 ± 0,02
Farga	0,20 ± 0,06	0,56 ± 0,03	4,34 ± 0,06	42,15 ± 1,20	1,57 ± 0,05	3,81 ± 0,09	0,07 ± 0,01	0,24 ± 0,03
Menuda	0,19 ± 0,02	1,19 ± 0,02	4,30 ± 0,08	17,33 ± 0,84	1,59 ± 0,05	2,14 ± 1,31	0,07 ± 0,01	0,23 ± 0,03
Serrana	0,20 ± 0,01	0,61 ± 0,02	4,43 ± 0,21	66,81 ± 0,66	1,50 ± 0,08	7,24 ± 0,09	0,12 ± 0,02	0,35 ± 0,01

Free acidity, Peroxide value, expressed as milliequivalents of active oxygen per kilogram of oil (meq O2/kg), and K232and K270 extinction coefficients calculated from the absorption at 232and 270 nm, respectively, were measured following the analytical methods described in European Regulation EEC 2568/91 and later amendments.

Both polar phenols content and tocopherol have been successfully correlated with olive oil stability [27-28]. In our study, as a result of oxidation, is predictable that oils coming from Serrana and Farga fruits have reduced its polar phenols and tocopherol contents.

By relating the results obtained in the color measurements with the results of the chemical analyzes we can observe a parallelism between the values of a* and b* and the level of primary and secondary oxidation of the EVOO after three years of storage. The most chemically modified EVOO (Farga and Serrana), with higher values of peroxides and K232 and K270 indexes coincide with the oils with lower chroma, higher values of a* and lower values of b*. On the other side, the EVOO of Cuquillo, the only EVOO that still suitable for consumption after three years of storage according to European Regulation, is the EVOO that has presented the higher chroma and higher b* value. No correlation has been found between the increase of the luminosity (L*) and the chemical quality of the EVOO.

The course of time worsens the quality of the EVOO. To delay at maximum the EVOO color degradation and chemical oxidation, it is recommended to store the oil in a fully filled container, well closed, in the dark and at a temperature between 13 °C and 18 °C and consume it before 18 months of bottling.

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