Oxidative stability of virgin olive oil

Virgin olive oil has a high resistance to oxidative deterioration due to both a triacylglycerol composition low in polyunsaturated fatty acids and a group of phenolic antioxidants composed mainly of polyphenols and tocopherols. Polyphenols are of greater importance to virgin olive oil stability as compared with other refined oils which are eliminated or drastically reduced during the refining process.

This paper covers the main aspects related to the oxidative stability of virgin olive oil during storage as well as at the high temperatures of the main processes of food preparation, i.e., frying and baking. Differences between oxidation pathways at low and high temperature are explained and the general methods for the measurement of stability are commented on. The compounds contributing to the oxidative stability of virgin olive oils are defined with special emphasis on the antioxidative activity of phenolic compounds. Finally, the variables and parameters influencing the composition of virgin olive oils before, during and after extraction are discussed.

Keywords: Virgin olive oil, stability, oxidation, phenolic compounds, storage, high temperature.

1 Introduction

Lipid oxidation has been recognised as the major problem affecting edible oils, as it is the cause of important deteriorative changes in their chemical, sensory and nutritional properties. Oxidation normally proceeds slowly at the initial stage and then a sudden rise occurs in the oxidation rate. The period of time which marks this change in the oxidation rate is called induction period or induction time. Oil quality is concerned with the present state of oil acceptability while oil stability is related to its resistance to future changes. Due to the evolution of oxidative degradation, the level of oxidation at early stages of the process gives poor information on the later oil behaviour and for this reason the evaluation of the oil stability towards oxidation is considered even more important than the extent to which the oil is oxidised [1].

Virgin olive oil, one of the few oils being consumed without any chemical treatment, has a high resistance to oxidative deterioration mainly due to two reasons; firstly, its fatty acid composition is characterised by a high monounsaturated-to-polyunsaturated fatty acid ratio and secondly, it contains a pool of minor compounds of powerful antioxidant activity among which polyphenols stand out [2]. Most of these compounds are eliminated or drastically reduced during the refining process and, consequently, are present in much lower amounts in edible refined oils than in virgin oils. However, it is also worthy to remark that even if virgin olive oil generally has a high resistance to oxidation, some minor compounds which are also eliminated during refining, i.e., free fatty acids and photosensitisers, are prooxidants and consequently will contribute to a high variability in the stability of virgin olive oils.

Olive oil is considered to be excellent for applications involving high temperatures. In particular, it fulfils all the fatty acid criteria of the stable healthful frying oils, i.e., being rich in monounsaturated fatty acids, low in saturated and polyunsaturated fatty acids, very low in linolenic acid and containing practically no trans fatty acids [3]. Moreover, olive oil is considered to be a premium frying oil with added advantages linked to its relatively low melting point. That means that it drains from the fried food easily leading to a low content of oil in the fried food [4].

This paper covers the main aspects related to the oxidative stability of virgin olive oil during storage as well as at the high temperatures of the main processes of food preparation, i.e., frying and baking. On the one hand, the compounds contributing to modify the susceptibility of virgin olive oils to oxidation are defined and on the other hand, the variables and parameters influencing the composition of virgin olive oils and, in turn, their stability towards oxidation at low and high temperatures are discussed. Previously, differences in the oxidation pathway due to the action of external variables and the general methods for the measurement of stability are commented.
2 External variables influencing the oxidative stability of olive oils

Despite the complexity of the oxidation process, the main reactions and variables involved in autoxidation, photooxidation and enzymatic oxidation are well known and documented [5-8]. Particularly, the excellent review written by Morales and Przybylski [9] is strongly recommended to readers interested in olive oil oxidation. Due to its recent appearance, comments in this section are limited to specific aspects which are useful to clarify the main differences introduced in the oxidation processes by the most important external variables influencing olive oil stability towards oxidation, i.e., oxygen concentration, temperature and light.

In the simplified scheme in Fig. 1 the main reactions involved in the oxidation of fats and oils are summarised. In olive oil oxidation, RH represents the triacylglycerol undergoing oxidation in one of its unsaturated fatty acyl groups. As can be observed, three triacylglycerol radicals - alkyl radicals (R') formed in the initiation reaction, alkylperoxyl radicals (ROO') originated by addition of oxygen and alkoxyl radicals (RO') formed by hydroperoxide decomposition - are involved in the formation of hydroperoxides (ROOH) and/or in the set of termination reactions. The latter lead to a high variety of compounds of different polarity, stability and molecular weight. Among them, three main groups of compounds stand out:

a) Compounds with molecular weights similar to those of the triacylglycerols (RH) undergoing oxidation in one of their unsaturated fatty acyl groups [10].

b) Volatile compounds with molecular weights lower than those of RH. These volatile compounds are produced by an alkoxyl radical breakdown [11].

c) Polymerisation compounds formed through interaction of two triacylglycerol radicals and thus, with molecular weights higher than those of RH [8, 12].

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. However, the main theoretical facts of interest to understand differences in stability at low and high temperatures can be summarised as follows:

a) The solubility of oxygen is high and alkylperoxyl radicals (ROO') are by far the most common radical species at atmospheric pressure and at low or moderate temperatures. Once the oil oxidation is initiated, reaction with oxygen is very rapid and ROOH are the major products originated. Under these conditions, ROOH formation rate is much higher than their decomposition rate. Compounds formed through termination reactions are only major products in the accelerated stage of the oxidation, i.e. at the end of the induction period when the concentration of the initial oxidisable substrate starts to decrease considerably [5].

b) The chemistry of oxidation at the high temperatures of food processes like baking and frying is much more complex since both thermal and oxidative reactions are involved simultaneously. As temperature increases, the solubility of oxygen decreases drastically al-
though all the oxidation reactions are accelerated. As the oxygen pressure is reduced, the initiation reaction becomes more important, the concentration of alkyl radicals (R•) with respect to that of alkylperoxyl radicals (ROO•) increases and polymeric compounds are formed through reactions mainly involving alkyl (R•) and alkoxyl (RO•) radicals [5].

These differences are in agreement with the following experimental results:

a) At low or moderate temperatures, the formation of oxidation compounds during the induction period is slow, ROOH are the major compounds formed and their concentration increases until the advanced stages of oxidation. Polymerisation compounds only become significant in the accelerated stage of the oxidation after the end of the induction period [13]. However, minor volatile compounds, in particular carbonyl compounds, are formed. They are of enormous sensory significance and may contribute to modify the oil flavour [14].

b) At high temperatures, the formation of new compounds is very rapid, ROOH are practically absent above 150 °C indicating that the rate of ROOH decomposition becomes higher than that of their formation and polymeric compounds have been formed since the very early stages of heating [15]. Also, the formation of significant amounts of non-polar dimeric triacylglycerols (RR), typical compounds formed in the absence of oxygen through interaction of alkyl radicals, is a clear indication of the low oxygen concentration [16].

2.2 Influence of light

Light is the third important external variable of great influence on photosensitised oxidation. It is well known that minor compounds (e.g. chlorophyll and derivatives in virgin olive oil) can be excited electronically due to the absorption of light. Subsequently, the excited molecule is able to transfer its excess energy to an oxygen molecule, giving rise to singlet oxygen, which reacts with olefinic double bonds by a concerted “ene” type mechanism. It was observed that singlet oxygen reacts about 1000–10,000 times faster than normal oxygen in a triplet ground state at ambient temperature. Therefore, the prevention of photoxidation during storage is of great importance to ensure a high oxidative stability [17].

Nevertheless, as temperature increases the influence of light on oxidation becomes progressively less pronounced being apparent that at the high temperatures of the processes for food preparation, light has no appreciable effect on the rate of oxidation [1].

3 Measurement of oxidative stability

The influence of temperature and oxygen concentration on the changes in activation energies and the mechanisms of oxidation reactions justify the need to consider two independent groups of methods for the evaluation of olive oil stability:

a) Methods for the prediction of the olive oil resistance to oxidation under storage conditions. In that case, information related to the oil shelf life will be obtained.

b) Methods for the prediction of olive oil performance at the high temperatures of processes of food preparation. Studies in this field are focused on frying because it is the only process in which the oil can be heated for a long time continuously or in which the oil can be used many times discontinuously.

3.1 Prediction of oxidative stability during oil storage

The objective of these methods is to gain a reasonable indication of the product shelf life in a short period of time. Also, they are useful to check the effect of minor compounds on the oil oxidative stability. A detailed description of the methods including their possibilities and limitations can be found in previous reviews [18, 19].

Tab. 1 summarises the methods applied for the measurement of oxidative stability at low and high temperature. In the upper part of the table, the four most common methods based on well-defined conditions to predict oil stability during storage are included.

3.1.1 Schaal oven test

This is the simplest accelerated test. Normally, 50 g of the oil are heated in a loosely sealed glass in an oven which is kept at a temperature of 63 °C. The sample is then examined periodically until the appearance of rancidity. Alternatively, peroxide values can be measured.

3.1.2 Active oxygen method (AOM)

This method measures the time in hours required for a sample of 20 g of oil or fat to attain a predetermined peroxide value (100 meq/kg) under the specific conditions of the test. The oil sample is placed in a standard glass tube and is aerated continuously at 98 °C. One-gram samples are withdrawn periodically for the peroxide value determination. The length of the period of time to reach a peroxide value equal to 100 meq/kg is assumed to be an index of oil resistance to rancidity, although the exact relationships between peroxide value, rancidity, and oxidative stability have not been firmly established so far.
3.1.3 Oil stability index (OSI)

This method is an automated replacement for the AOM. Rancimat and Oil Stability Instrument, both of them working according to the same principle, are the two commercially available instruments for this analysis.

Applying this method, a stream of purified air is passed through a sample of 5 g of oil or fat that is held at a constant temperature and air flow. The effluent air from the oil sample is then bubbled through a vessel containing deionised water. The conductivity of the water is continuously monitored. The effluent air contains volatile organic acids swept from the oxidising oil, which increase the conductivity of the water as oxidation proceeds. The OSI is defined as the time, expressed in hours, that is needed to reach the maximum change of conductivity. Although it may be run at many temperatures, the range 100-140 °C was found to be suitable for most oils.

3.1.4 Oxygen uptake method (Oxidograph)

Using this method, 5 g of oil are placed in a closed vessel also containing 100 ml oxygen. The pressure reduction in the vessel which is due to the oxygen consumption is monitored continuously. The method measures the induction time (in hours) as the point of maximum change in the rate of oxygen uptake. Temperatures around 100 °C are commonly used.

As can be observed in Tab. 1, AOM and OSI are standard methods and they are the most widely applied ones to virgin olive oils. Furthermore, Tab. 1 shows that the rate of oxidation which increases exponentially with absolute temperature is the preferred parameter to accelerate oxidation in the main tests available. Even more, it is interesting to remark that the methods have excess amounts of air or oxygen in common.

The major drawback attributed to the tests at around 100 °C is that their results could be unreliable because the mechanism of oxidation changes from the ambient temperature to that of the test [20]. However, from the point of view of the prediction obtained, two other aspects are also worth mentioning:

1. As observed in Tab. 1, oxidative stability methods give information on oil stability based on different points of the oxidation process, i.e., evaluation of the induction period by a sudden change in conductivity or oxygen depletion in the automated methods, the appearance of rancidity in the Schaal oven test, or the time to reach a peroxide value equal to 100 meq/kg oil in the AOM. Nevertheless, the end of the acceptable oil shelf life is commonly based on the values of specific parameters established in official regulations. For example, virgin olive oils with peroxide values higher than 20 meq/kg cannot be commercialised as edible oils. That means that stability measurements not only involve differences in temperature but also in the oxidation level measured.

2. Even more important is the fact that the oil shelf life may vary greatly depending on external parameters other than temperature. Oil storage in light or dark as well as differences in oxygen availability due to packing and storage conditions are important enough to ex-

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Tab. 1. Main methods for the measurement of oil stability†.

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<th>Objective</th>
<th>Test</th>
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| Prediction of stability during storage | Oil Stability Index (OSI) | Increase of conductivity is recorded automatically and IT calculated | • Standard AOCS Method Cd 12b-92  
• 100-140 °C  
• Bubbling air |
| | Active Oxygen Method (AOM) | Time to reach PV = 100 meq/kg oil calculated | • Old Standard AOCS Method 12-57  
• 98 °C  
• Bubbling air |
| | Oxydograph | Reduction of oxygen pressure is recorded automatically and IT calculated | • High initial oxygen pressure  
• Test is performed around 100 °C  
• Also applied to foods |
| | Schaal test | Rancidity appearance and/ or peroxide value | • 63 ± 0.5 °C  
• High surface-to-oil volume ratio to guarantee oxygen availability |

† IT - Induction period; PV - Peroxide value.
pect high differences in the oil resistance to oxidation. However, any method applied to predict oil shelf life would give a single value representing the stability under some given conditions.

In consequence, the objective of these tests is to provide useful comparative data on the susceptibility of different oils to oxidation under certain conditions rather than an accurate value representative of the oil shelf life. In this respect, any of the methods proposed is useful and, among them, standard methods would be preferable to facilitate the intercomparison and understanding of the thousands of data on oil stability given in different reports.

Other oxidation conditions involving light, enzyme or metal catalysts [20-22], or the addition of radical initiators [23, 24] were applied although no standard conditions have been defined yet. Different authors used different versions and the comparison of the results obtained is difficult. Even more, when the results were compared with those obtained by the standard methods (either AOM or OSI), correlations were highly variable [21, 22].

Finally, AOM or OSI have been the most applied methods in studies on virgin olive oil. Values for fresh oils obtained at 100 °C are, as expected, highly variable ranging from only a few hours to more than one week [19, 25-27]. However, although great differences in stability are found due to agronomic and environmental variables, when grouping the samples, the highest stabilities in each group correspond to extra virgin olive oils and the lowest to lampant oils of high free fatty acid content.

3.2 Prediction of oil performance during frying

The frying process is influenced by a large number of variables among which the type of process, i.e., continuous or discontinuous, the surface-to-oil volume ratio, the food, the temperature and the oil selected, are of particular relevance [15]. Due to the difficulties encountered in defining and/or in controlling such variables and the additional strong interactions between them [28], it is not easy to replicate results from different laboratories. Moreover, the conclusions drawn from data obtained under apparently similar conditions might differ greatly. Hence, development of suitable methods based on standard conditions giving information on oil heat stability is of major interest.

Two simple procedures summarised in the lower part of Tab. 1 were recently proposed to evaluate the performance of fats and oils at frying temperatures and to check the efficacy of minor compounds influencing stability at high temperatures.

In the first procedure, the oil sample (8 g) is heated at 180 °C for 10 h taking some of the advantages provided by the Rancimat apparatus such as standard vessels, temperature correction and temperature homogeneity in all vessels [29]. Quantification of polymers and/or polar compounds were considered the most useful analytical criteria for comparing different samples as specific values for discarding frying fats and oils have been established in the official regulations of many countries [30]. Repeatability was very good as the evaluation of samples in triplicate gave coefficients of variation <5% for polymers and <6% for polar compounds.

In the second procedure, 20 g of sample supplemented with 1 g of silica gel containing 10% water were heated in a standard vessel (4 cm i.d.) at 170 °C for 2 h. After quantification of polymers, results are expressed as 100/polymer (%) and consequently, the higher the value obtained, the more stable the oil [31]. The method was applied to different oils and a good correlation with sensory data was found.

The main advantage of the methods evaluating stability at high temperature is that it is not necessary to use conditions accelerating the reactions involved as the temperature is similar to that applied in the process. On the other hand, the concentration of oxygen is limited to that which is naturally absorbed through the vessel surface. Consequently, the main drawback attributed to those methods predicting the oil shelf life is eliminated.

General application of any of the methods would undoubtedly be useful to clarify differences in stability between oils and between batches of the same type of oil in discontinuous frying. However, ring tests would be necessary to know the reproducibility of the results obtained in different laboratories. Also, taking advantage of instruments of common use such as Oil Stability Instrument or Rancimat as the best standardised heating system would contribute to improve reproducibility and to have comparative results of similar utility to those obtained for the prediction of oil stability during storage.

The performance of olive oil at frying temperatures has been studied by heating the oil in the absence of food or by simulating domestic frying. Due to the different variables applied (i.e., continuous or discontinuous heating, different surface-to-oil volume ratio, different temperatures and foods, addition of fresh oil or not, etc.), the oil degradation expressed as polar compounds or polymers ranges from values close to those of the fresh oil to those corresponding to oils unsuitable for human consumption. Nevertheless, when different oils were compared, olive oil was considered to be the most stable liquid fat with added value due to its nutritional properties [3, 32-35].

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4 Compositional factors influencing virgin olive oil stability

Virgin olive oil is mainly composed of triacylglycerols (around 95%), a minor variable amount of free fatty acids and minor glycericidic compounds – partial glycerides, phospholipids and oxidised triacylglycerols – and around 1% of unsaponifiable constituents of varied structure and polarity [2, 36].

Most of the groups of minor compounds are reported to have either a beneficial or detrimental effect on oil stability although the positive contribution of the primary antioxidants present in the unsaponifiable fraction is the major determinant in the resistance of virgin olive oil to oxidation.

4.1 Major compounds: triacylglycerols

The fatty acid and triacylglycerol composition of virgin olive oil differs considerably depending mainly on latitude, climate, variety and stage of maturity of olives. The percentages of the two major unsaturated fatty acids, oleic and linoleic acids, vary from 55 to 83% and from 3.5 to 21%, respectively [36]. Considering the high influence of the fatty acid composition on the stability of edible fats and oils at any temperature, some authors investigated whether these differences are important enough to make a significant contribution to the oil resistance to oxidative deterioration. In this respect, Salvador et al. found low correlation coefficients between fatty acids and oxidative stability measured by Rancimat in virgin olive oils from successive crops [37]. Aparicio et al., analysing 79 samples of virgin olive oils cv. Pical y Hojiblanca, reported a contribution of 24% of the fatty acid composition to the oxidative stability measured by Rancimat. This contribution was much lower than the one of 51% found for polyphenols [27]. This low contribution was expected since previous studies revealed the great influence of processing variables on oil stability. Nevertheless, processing variables do not contribute to modify the major compounds in virgin olive oil. This would imply that oils with identical fatty acid composition may have different resistance to oxidative degradation and, consequently, that major compounds have a minor and variable participation in olive oil stability.

4.2 Minor unsaponifiable compounds

4.2.1 Phenolic compounds: polyphenols and tocopherols

Polyphenols and tocopherols are the two main groups of phenolic compounds acting as primary antioxidants to inhibit oxidation in virgin olive oils. They mainly act as chain breakers by donating a radical hydrogen to alkylperoxyl radicals formed during the propagation step of lipid oxidation and subsequently forming a stable radical (A•) through the well-known reaction:

$$\text{ROO}^- + \text{AH} \rightarrow \text{ROOH} + \text{A•}$$

While tocopherols constitute the lipophilic antioxidant group and are noted for their effective inhibition of lipid oxidation in all vegetable oils, polyphenols, the active hydrophilic group, are only present in significant amounts in virgin oils since they are practically lost during refining. The composition of polyphenols is highly complex. Tyrosol, hydroxytyrosol, simple phenolic acids and esterified derivatives of tyrosol and hydroxytyrosol are the most representative compounds [38, 39]. In the last years, a great effort was made to identify new relevant components [40-43] since it is assumed that they are responsible for the differences found between virgin and refined oils regarding both nutritional properties and stability. Unfortunately, there are different analytical methods varying in extraction and separation procedures as well as in the expression of results, which makes the comparison of the quantitative results obtained difficult. In this regard, a common effort to establish a standard method would enormously simplify the data comparison.

Polyphenols and in particular, ortho-diphenols are reported to be the highest contributors to oxidative stability in virgin olive oils for many years. Most of the papers studying the influence of different variables on changes in polyphenols also determined the stability measured by the Rancimat and good correlation coefficients between the two parameters are found in all the studies [25, 27, 39, 40, 44-48].

With respect to tocopherols, the major constituent in olive oils is α-tocopherol, β- and γ-tocopherol are only present in minor amounts. The tocopherol content is highly variety-dependent although usual values reported for extra virgin oils vary between 100 and 300 mg/kg [49]. In virgin olive oils, they compete with polyphenols at the early stages of oxidation and their contribution to virgin olive oil stability is considered to be of minor importance with respect to that of polyphenols. However, it is important to mention that, apart from their action as lipid radical scavengers, they also inhibit the photooxidation by reacting with singlet oxygen either by physical quenching or by chemical reactions [50]. Thus they contribute to an increase in the oxidative stability of oils during storage in the presence of light [51].

Studies on individual compounds added to refined oils or oils stripped of antioxidants demonstrate the major contribution of hydroxytyrosol in the effective inhibition of oxidation [46, 47, 52, 53]. Servili et al. found that the oleo-
Changes in the phenolic compounds of virgin olive oils during storage are also reported. Cinquanta et al. studied the evolution of simple phenols during 18 months of storage in the dark [54]. They found a great increase in the tyrosol and hydroxytyrosol contents due to hydrolysis of their complex derivatives in a first stage, and a rapid loss of hydroxytyrosol as compared with that of tyrosol at the end of the storage period. In a recent study, Pagliarini et al. [55] presented interesting results on the influence of chemical compounds on the virgin olive oil shelf life under different commercial conditions. Experimental data were processed by multivariate analyses to deduce the most significant parameters for predicting shelf life. Hydroxytyrosol, tyrosol, tocopherol contents as well as Rancimat stability were found to be significant. Regression analyses for the significant parameters indicated that the formation of hydroxytyrosol and tyrosol during storage followed a pseudo-zero-order kinetic and, the decrease in tocopherol content and Rancimat stability followed a pseudo-first-order kinetic. Unfortunately, due to the hydrolysis of phenol derivatives, the relative losses of simple phenols and tocopherols due to oxidation are unknown.

Nevertheless, the activity of phenolic compounds decreases drastically at the high temperatures of the food preparation processes. Firstly, since reactions are very rapid and the concentration of ROOH decreases rapidly with increasing temperature, it follows that phenolic antioxidants will become less effective with increasing temperature. Secondly, since ROOH are the products of the reaction of alkylperoxy radicals with the antioxidant, their immediate decomposition would even lead to chain propagation rather than chain breaking under these conditions. Finally, side reactions of oxidation or thermal alteration of phenolic compounds have also to be considered.

Since many years, it has been known that the loss of tocopherols is more rapid at high temperatures as the degree of oil unsaturation decreases. But then the opposite is true at low temperatures and around 100 °C which are the conditions used for oxidative accelerated tests [56]. Concomitantly, it was found that tocopherols are exhausted at lower levels of oxidation in monounsaturated oils than in polyunsaturated systems. These results suggest an antioxidant mechanism dependent on both temperature and the loss of tocopherols by competing reactions [57, 58].

With respect to polyphenols, two studies on the changes of primary antioxidants in virgin olive oils also indicated their rapid degradation at frying temperatures. On the one hand, Beltran Maza et al. [59] analyzed the changes in tyrosol, hydroxytyrosol and α-tocopherol during 25 frying operations of potatoes. The three natural antioxidants had different evolutions under the assayed conditions. Hydroxytyrosol was only detected until the sixteenth frying operation, tyrosol degradation was slower and it was detected until the end of the experiment and tocopherol degradation followed a pseudo-zero-order kinetic. On the other hand, Pellegrini et al. reported the evolution of total phenols and α-tocopherol at frying temperatures (160-190 °C) and after heating times typical of domestic frying conditions (0.5-2 h). Results from samples with a similar content of α-tocopherol and increasing contents of polyphenols suggest that polyphenols are effective stabilizers of α-tocopherol during heating as the percentages of α-tocopherol remaining in the oil were higher with an increasing concentration of polyphenols. However, in all the samples significant degradation of phenolic compounds occurred. Unfortunately, no parallel data on triacylglycerol degradation were given [60].

Summing up, it may be said that the inhibiting action of phenolic antioxidants on oxidation seems to be well documented. Nevertheless, selective research remains to be done on standardisation of analytical techniques, on the degradation routes of phenolic compounds under different conditions and on explaining the interactions between phenolic compounds and other minor compounds exerting a variable influence on olive oil oxidation both at low and high temperatures.

### 4.2.2 Squalene

Squalene is the major constituent of the unsaponifiable matter in olive oil with a concentration of up to 40% by weight. Psomiadou and Tsimidou reported interesting results of the squalene action at low or moderate temperatures. Primarily, they found that squalene plays a confined role in virgin olive oil stability due to the presence of more active compounds such as polyphenols and tocopherols. Additionally, they also detected that in the absence of active antioxidants, squalene showed no effect at the temperatures of the OSI test but a moderate antioxidant activity at low temperatures in the dark [61].

At a high temperature, a moderate antioxidative action was found in model systems [62] although the antioxidative mechanism is not well understood. However, studies on the action of the unsaponifiable fraction of olive oil conducted either at low [63] or high temperatures [64] indicate a combined mode of action of α-tocopherol and squalene as chain-breaking antioxidants. It was suggest-
ed that $\alpha$-tocopherol could be regenerated from the tocopheroxyl radical by squalene [63].

### 4.2.3 Sterols
Sterols are also major constituents of the unsaponifiable fraction and their content is in the range of 180-265 mg/100 g oil [65] corresponding to around 20% of the unsaponifiable matter. They seem to be ineffective at low temperatures and under the conditions of accelerated tests although their influence on oil stability at high temperature where they act as inhibitors of polymerisation reactions was demonstrated in a considerable number of studies [66-68]. Detailed experiments showed that $\beta$-sitosterol, campesterol and stigmasterol were ineffective, while $\alpha$-avenasterol and other related sterols such as $\Delta 7$-avenasterol and citrostadienol were active at high temperatures. The mode of action of these phytosterols possessing an ethylidene side chain is not fully elucidated although formation of allylic-free radical at carbon C29 followed by isomerisation to a relatively stable tertiary free radical was proposed [68].

### 4.2.4 Chlorophylls and derivatives
Chlorophylls and their derivatives are present in olive oils in variable amounts mainly in the form of degradation products, such as pheophytins. The content of chlorophylls and their derivatives depends on the stage of olive maturity decreasing continuously from the beginning to the end of the olive picking period [69].

In the presence of light, chlorophylls and their derivatives are the most active promoters of photosensitised oxidation in virgin olive oil greatly contributing to their susceptibility to oxidation [70]. The influence of the exposure of virgin olive oil to light was studied in detail by Rahmani and Saari Csallani [71] at 2 °C and 40 °C. They showed the enormous effect of increasing intensities of fluorescent light on the rate of oxidation. Results were attributed to pheophytin A, the only chlorophyll derivative present in the initial sample. Practically no differences in the oxidation levels or in the parallel losses of phenolic antioxidants due to the temperature were found when light was present. However, as expected, differences due to the temperature were found in the dark. On the other hand, Gutiérrez et al [72] found out that the addition of chlorophyll to a virgin olive oil containing natural pheophytin A did not result in an increase in the prooxidant action under light conditions. This result is founded on the fact that no differences in oxidation levels or in Rancimat stabilities remaining after different storage period were found. These findings suggest that once the compounds are present in sufficient amounts, the increase in their concentration is of minor importance. These photosensitisers may also show slight antioxidant effects on the oils in the dark probably by donating hydrogen to break the free-radical chain reactions [72, 73].

### 4.2.5 Carotenoids
Spanish olive oils contain 3.1-9.2 mg/kg of carotenoids [74]. Carotenoids and specially $\beta$-carotene are strong protectors against photosensitised oxidation acting as singlet oxygen quenchers [75]. In the study reported by Rahmani and Saari Csallani, the activity of $\beta$-carotene as a strong natural inhibitor of virgin olive oil photoxidation at 2 °C was deduced from the rate of the disappearance of $\beta$-carotene in the presence of light [71]. In the absence of light, carotenoids and their oxidation products may act as prooxidants in vegetable oils [76]. In a study by Wagner and Elmadfa, addition of $\beta$-carotene in various concentrations resulted in prooxidation of olive oil when heated at 120 °C but no effects were found when $\beta$-carotene was added to sunflower oil or linseed oil [77].

At frying temperatures, the action of carotenoids on olive oil stability was not studied in detail. However, $\beta$-carotene was not effective in protecting oils during thermoxidation [78] and, in frying experiments with red palm oil of high carotene content, the total loss of $\beta$-carotene took place by the fourth frying operation [79].

### 4.2.6 Metals
Many reports describe the deleterious effect of trace amounts of metals on the oxidative stability of edible oils. Transition metal ions promote free-radical formation because a single electron transfer occurs during their change of oxidation states. Metals enhance the formation of radicals in both initiation and hydroperoxide decomposition steps [8].

In virgin olive oil, traces of Fe and Cu may originate from the soil and fertilisers or from the contamination of the processing equipment and storage containers. Concentrations of Fe and Cu reported for virgin olive oil range between 0.5-3.0 and 0.001-0.2 mg/kg, respectively [80]. Other metals reported to be present in virgin olive oil are Cr, Mn, Sn, Ni and Pb, whose concentrations did not exceed a few µg/kg [81].

It was shown that virgin olive oil had a lower stability after storage in contact with carbon steel than when stored in the absence of metals [82]. Recently, Angerosa and Di Giacinto found that traces of Mn and Ni in virgin olive oil may cause changes in colour and flavour due to the partial destruction of chlorophylls and polyphenols. Nevertheless, the increase in the content of both metals did not affect the oxidation indices significantly during storage.
However, detailed studies to establish the mode of action of natural metals on virgin oil stability have not been performed so far. As chelators inhibit the prooxidant effect of natural metals, it would be interesting to know if some minor natural chelating agents like phospholipids, polyphenols or proteinaceous materials exert a significant protective action in virgin oils against metal-catalysed oxidation.

4.3 Minor saponifiable compounds

4.3.1 Free fatty acids

A prooxidant effect of free fatty acids on edible oils was demonstrated by the addition of different fatty acids to purified substrates [85, 86]. The action has been attributed to the free carboxylic group since no effect was found when non-acidic lipophilic compounds were added [86]. In virgin olive oils the action of fatty acids was studied starting with samples from the same variety and different levels of hydrolytic degradation. The results showed clearly that the stability measured by Rancimat decreased with an increasing level of free fatty acids [87]. Also, Frega et al. studied the influence of oleic acid addition to virgin olive oils on the oxidative stability measured by Rancimat at 110 °C. They found a prooxidant effect dependent on the free fatty acid concentration in filtered virgin olive oils. Surprisingly, the opposite behaviour was found when the oil was not filtered. According to the authors, suspended-dispersed materials in cloudy virgin oils exert such a beneficial effect that avoidance of filtration would be desirable in order to extend the oil shelf life [88]. At frying temperatures, the presence of fatty acids has been shown to catalyse both oxidation and hydrolysis of triacylglycerols [89]. Even more important is their contribution to the decrease of the smoke point due to their partial volatilisation. Thus they contribute to the decrease of the shelf life of frying fats and oils in both catering and industrial frying processes [90].

4.3.2 Phospholipids

Virgin olive oils contain phospholipids in the range of 40-135 mg/kg [91]. The antioxidant activity of phospholipids was attributed to their capacity to chelate metals and thus inactivate their prooxidant effect. Moreover, they can act as synergists with phenolic compounds and tocopherols contributing to enhance their antioxidant activity [92]. At a high temperature their beneficial action was also demonstrated although they may cause foaming and darkening and thereby decrease the oil shelf life in practice. However, it is generally accepted that their presence at a low concentration (<0.01%) does not exhibit an adverse effect on the oil colour and foaming, but can improve the frying stability of oils [89].

4.3.3 Other minor glyceridic compounds: Partial glycerides and oxidised triacylglycerols

The presence of partial glycerides in virgin olive oil is due to either incomplete triacylglycerol biosynthesis or to hydrolytic reactions. The slight prooxidative action of partial glycerides and oxidised triacylglycerols was demonstrated by addition of variable amounts to refined oils [93]. Miyashita and Takagi analysed the evolution of oxidation in different independent substrates, i.e. mono-, di- and triacylglycerols and they found that the increase in the number of esterified positions in the triacylglycerol resulted in a higher resistance to oxidation [94]. At frying temperatures, a similar prooxidative activity was found although from the point of view of the oil shelf life, contents of monoacylglycerols exceeding 0.4% should be avoided since they may cause excessive foaming giving an indication that the oil has reached the end-point and should be discarded [89].

Summing up, it may be said that the objective of this section was to give a general idea of the effects of the compounds present in virgin olive oils on their stability towards oxidation. However, because of the complexity of the oxidation process itself, the high number of groups of compounds of recognised and unrecognised influence on oxidation, their complementary or opposite actions, the influence of their concentration on their activity and even the difficulties in their accurate quantification, it is not astonishing to find controversial results in some studies. Also, variable contributions to the oxidative stabilities found for the same variables in different studies would indicate both the influence of the origin of the samples and of their different quality. On the other hand, results extracted for a specific group of samples are sometimes extended to the virgin olive oil population in general and thus they might be the reason for erroneous deductions.

As an example of the difficulties involved in oxidation studies and of the possibilities of reaching definite conclusions, Tab. 2 shows the stabilities at 100 °C and at 180 °C of 3 extra virgin olive oils with different polyunsaturated-to-monounsaturated fatty acid ratios, before and after elimination of polar compounds, and after adding 500 mg/kg α-tocopherol to the stripped oils. The results obtained show the complexity of the stability studies. As can be observed in the initial virgin oils, Rancimat stability was highly variable and seems to be dependent on the only variable included in the table, i.e., the ratio C18:1/C18:2 in the samples. However, the Rancimat stability decreased...
drastically reaching similar values for all oil samples after elimination of the polar fractions, i.e., compounds with a higher polarity than that of triacylglycerols (including all the minor compounds with the exception of hydrocarbons, waxes and sterol esters). Besides, the stability at high temperatures also decreased significantly. Apparently, these results indicate both the enormous importance of the eliminated fraction and the minor influence of the triacylglycerol composition on the virgin oil stability. They also point out that squalene present in the nonpolar fraction did not protect the major compounds. However, after adding a similar amount of \( \alpha \)-tocopherol to each stripped oil, the influence of the triacylglycerol composition became evident. Addition of \( \alpha \)-tocopherol to the stripped oils did not only result in an increased stability of the three oils at both temperatures, but also in an increase in efficacy parallel to the increase in the C18:1/C18:2 ratio. These results show the difficulties in reaching definite conclusions on the action of compounds influencing oxidation even in simplified systems. Furthermore, they demonstrate how results may conduct to misleading interpretations.

### 5 Variables influencing the composition of olive oil

The final composition of virgin olive oils is the result of a high number of variables taking effect from the oil formation in the olive tree to the status of the oil at consumption. Some of these variables have important effects on the concentration of compounds modifying the stability towards oxidation and are divided into three groups in this section: those acting before oil extraction, those acting during oil extraction and finally, those acting after oil extraction during storage.

#### 5.1 Variables acting before oil extraction

Numerous factors such as olive variety, environmental, climatic, soil and cultivation conditions, age of the tree, olive ripeness, olive health, etc. are involved in the composition differences in virgin olive oil during its formation in the fruit.

Inevitable differences are found in the stabilities and concentrations of minor antioxidative compounds in extra virgin oils obtained from different varieties and growing areas [27, 37, 95]. For example, great quantitative differences in the concentrations of polyphenols are reported between small-fruit and large-fruit varieties [96]. Thus, good-manufacturing practices to maximise both quality and stability of the virgin oils obtained from each variety is of prior importance. In this respect, the influence of olive ripeness on the composition and stability of virgin olive oil was the subject of numerous investigations. As the ripeness index increased the amounts of phenolic compounds decreased thus resulting in lower oil oxidative stabilities [37, 54, 69, 97-99]. Also chlorophylls and carotenoids decreased drastically while polyunsaturated fatty acids and avenasterol increased [37, 69]. These great changes stress the need of a careful selection of the harvesting time to obtain compatible high oil yields, excellent organoleptic characteristics and high stability.

### Tab. 2. Influence of minor polar compounds on virgin olive oil stability.

<table>
<thead>
<tr>
<th>Sample</th>
<th>C18:1/C18:2</th>
<th>Rancimat 100 °C [h]</th>
<th>Polymers (10 h at 180 °C) [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil 1</td>
<td>15.5</td>
<td>112 ± 2.3†</td>
<td>3.8 ± 0.25†</td>
</tr>
<tr>
<td>Nonpolar fraction from oil 1</td>
<td>15.4</td>
<td>2.5 ± 0.05</td>
<td>9.2 ± 0.20</td>
</tr>
<tr>
<td>+ 500 mg/kg ( \alpha )-tocopherol†</td>
<td>15.6</td>
<td>38.9 ± 0.20</td>
<td>6.7 ± 0.35</td>
</tr>
<tr>
<td>Oil 2</td>
<td>9.3</td>
<td>68 ± 1.2</td>
<td>4.8 ± 0.10</td>
</tr>
<tr>
<td>Nonpolar fraction from oil 2</td>
<td>9.5</td>
<td>2.4 ± 0.00</td>
<td>9.5 ± 0.15</td>
</tr>
<tr>
<td>+ 500 mg/kg ( \alpha )-tocopherol†</td>
<td>9.3</td>
<td>25.2 ± 0.25</td>
<td>7.1 ± 0.05</td>
</tr>
<tr>
<td>Oil 3</td>
<td>5.5</td>
<td>42.1 ± 0.80</td>
<td>6.5 ± 0.20</td>
</tr>
<tr>
<td>Nonpolar fraction from oil 3</td>
<td>5.5</td>
<td>2.1 ± 0.10</td>
<td>9.5 ± 0.05</td>
</tr>
<tr>
<td>+ 500 mg/kg ( \alpha )-tocopherol†</td>
<td>5.4</td>
<td>20.8 ± 0.20</td>
<td>7.6 ± 0.25</td>
</tr>
</tbody>
</table>

† Mean ± SEM (n = 2).
‡ Added to nonpolar fractions.
On the other hand, Motilva et al. [99] found that deficit irrigation applied to olive trees increased oil stability and polyphenol concentration at different picking dates. Also, the health state of olives has a considerable effect on oil stability. Particularly, infestation by Dacus Oleae must be controlled in order to ensure a prolonged oil shelf life [100, 101].

During and after harvesting, olives can be bruised facilitating the contact of the lipases with their substrates and the growth of microorganisms contributing to the lipolysis. Further, the conditions in the fruit pile during a long storage such as rising temperature and humidity favour both the hydrolytic and oxidative degradations. Studies on olive storage showed that acceptable olive oils could be obtained after one-month storage at 5 °C [102, 103]. However, conditions promoting an increasing free fatty acid content must be avoided since free fatty acids not only accelerate oxidation and thereby decrease oil stability but are also conductive to the hydrolysis of complex phenols which could result in a minor phenols concentration in the oil due to the high solubility of simple phenols in water [104].

5.2 Variables acting during the extraction of olive oil

Since the introduction of continuous systems for olive oil extraction a considerable number of studies have been directed to investigate the influence of the different stages of the process on the quality and stability of virgin olive oils.

5.2.1 Crushing

In continuous systems, the stone mills were replaced by metal crushers, usually hammer crushers. These mills may present the drawback of incorporating metal traces and air into oils, which favour lipid oxidation. However, Alloggio and Caponio found that oils obtained from hammer mills had a higher polyphenol concentration than oils from stone mills [97] and Ranalli et al. reported that double milling, combining hammer crusher and stone grinding, improved oxidative stability in comparison with single milling with a hammer crusher [105].

5.2.2 Olive paste preparation

Application of different temperatures during malaxation resulted in an increase in polyphenol extractability and oil stability with increasing paste temperature [106, 107]. On the contrary, dilution of the paste with water and increasing malaxating times led to the opposite effect due to the retention of phenolic compounds in the water phase [106].

Also, the effect of coadjuvant addition on olive oil stability was reported. Thus, addition of micronised talc to the olive paste to break emulsion resulted in a significant increase in oil stability and a slight decrease in oxidised triacylglycerol levels [108]. Similarly, the addition of enzymes with pectinolytic, cellulolytic and/or hemicellulolytic activities resulted in oils with a higher content of phenolic compounds [109-113].

5.2.3 Extraction procedure

The comparison between the different extraction systems was the subject of numerous investigations mainly varying in the samples selected and in the compounds measured. Results from different studies agree in two points; that the extraction system does not modify the fatty acid composition and that, when water was added during oil extraction, a lower concentration of phenolic compounds is found in the oil. Di Giovacchino et al. [114] found that oils obtained by pressure extraction had higher polyphenol and ortho-diphenol contents and, consequently higher stabilities towards oxidation than those obtained by centrifugation. That was attributed to the addition of warm water to olive pastes in the 3-phase centrifugation system. Similar results were found when the two centrifugation systems were compared. Virgin oils obtained by the 2-phase procedure, which does not require water addition, showed a higher resistance to oxidation than those obtained by 3-phase extraction [108, 115-117]. Furthermore, those oils showed not only higher polyphenol and ortho-diphenol contents but also a higher content of tocopherols and a lower concentration of pigments [118, 119]. Sciancalepore et al. [120] found that virgin oils obtained by cold percolation had a higher oxidative stability than those from 2-phase extraction system. Besides, oils obtained by percolation showed a higher polyphenol content and lower free acidity, peroxide value and UV absorption.

5.2.4 Filtering

Filtered oils are less stable than cloudy oils containing suspended and dispersed materials. Apparently, due to its composition, these suspended materials play a stabilising role by acting as antioxidants and/or as a buffer against increasing acidity. Thus, filtering should be avoided to increase oil shelf life [88, 121].

5.3 Variables acting after oil extraction

Once the olive oil has been extracted, oxidative deterioration can be influenced by external variables among which oxygen availability, temperature, light and possible metal contamination during storage stand out.
Pérez Cerezal et al. reported that olive oil stored in iron tanks can be oxidised rapidly if they are not coated internally with epoxy resins [122]. They also compared the differences in oxidative deterioration between virgin olive oils stored in an iron tank and those stored in a polyester-glass fiber tank. After 10 months, the former had undergone a significant oxidative deterioration while the oxidation extent of the latter was as low as that found for the control oil stored in glass bottles at 4 °C [123]. In this respect, stainless steel is considered the most appropriate material for tanks in order to avoid the presence of detrimental factors during storage, i.e., light and metal contamination. Additional protection with inert gas to decrease oxygen concentration would be the complementary measure to maintain a high oil stability [124].

The influence of packing materials on olive oil stability was also studied in detail under different commercial conditions [55, 125-129]. As expected, results from long-term storage studies indicate that impermeability to air and protection from light increase the oil shelf life significantly. At present, virgin olive oil is usually commercialised in glass transparent bottles since consumers like to see the oil that they purchase. Consequently, the lack of protection from light is the predominant factor that can accelerate oxidation by catalysis of radical initiation or, in the presence of photosensitisers, by formation of singlet oxygen.

<table>
<thead>
<tr>
<th>Tab. 3. Variables influencing olive oil composition and stability.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main variables</strong></td>
</tr>
</tbody>
</table>
| Before olive oil extraction | Variety and environmental, climatic and soil conditions | • Fixed parameters defining unavoidable differences in virgin olive oil composition | • Avoid olive infection  
• Suitable ripening at harvesting  
• Avoid fermentation during storage |
| | Cultivation conditions | • Irrigation deficit increases polyphenol content |
| | Degree of ripeness | • Ripeness increases polyunsaturated fatty acids and decreases polyphenol and pigment contents. |
| | Olive infection | • Decrease in polyphenols and increase in free fatty acids |
| | Olive storage | • Storage promotes fermentation, increasing free fatty acids and partial glycerides |
| During olive oil extraction | Milling | • Hammer crushing increases polyphenol concentration as compared with stone mills  
• Talc and enzyme addition slightly increase the polyphenol content |
| | Olive paste preparation | • Polyphenols increase with temperature and decrease with time and water addition |
| | Oil extraction system | • Percolation gives the highest polyphenol content  
• Addition of water in 3-phase centrifugation system decreases the polyphenol concentration |
| | Filtering | • Suspended and dispersed materials act as oil stabilizer |
| After olive oil extraction | Oil storage | • Certain materials promote metal contamination  
• Storage in the dark  
• Minimum headspace  
• Impermeable packing |
| | Retail packing | • Light promotes photoxidation |
Summing up, it may be said that Tab. 3 summarises the main variables and parameters influencing virgin olive oil stability towards oxidation in the different stages, their main effect on olive oil composition and the practical measures to maximise stability by three possible obvious ways:

1. Maximising the concentrations of compounds exerting an antioxidative action. In particular high contents of phenolic compounds would be a guarantee for high stability.

2. Minimising the concentrations of compounds exerting a prooxidative action. In this respect, the content of free fatty acids being as low as possible would contribute to a high resistance to oxidation.

3. Avoiding the external conditions as much as possible which promote the oxidative deterioration. In this connection the presence of light and high concentrations of oxygen would be the most deleterious.

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References

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