Chapter 4

Stabilization of Extra-Virgin Olive Oil

Lorenzo Guerrini and Alessandro Parenti

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64401

Abstract

The conservation of virgin olive oil quality during its shelf life could be considered a key issue for olive oil industry. To improve the product stability, virgin olive oils should not be stored with considerable amounts of suspended solids and water. The latter have to be removed from oil musts. The chapter reviews the main spread technologies and those recently proposed for the removal of suspended solids and the water from extra-virgin olive oils. These technologies are described from an engineering perspective, and their effects on product quality during storage are discussed.

Keywords: shelf life, biophenols, quality, vertical centrifuge, filtration

1. Introduction

Virgin olive oil (VOO) is a product with a relatively long shelf life. Producers consider 12–18 months as the optimal period from production to consumption. However, the olive oil produced in a harvest season is usually consumed before the following season [1]. Furthermore, at the beginning of a harvest season, the VOO from the previous season undergoes a serious price reduction. During the storage, VOO undergoes a large number of changes in its chemical and sensory characteristics. For this reason, many solutions have been proposed to retain VOO quality and extend its shelf life.

2. Oil must characterization

The VOO coming from the decanter centrifuge, the oil must, is veiled. Decanters separate the oil from olive paste and water, and the centrifuge settings affect the VOO cloudiness and the yield. Oil must is an intermediate product of the VOO production process, and it has to be filtered or precipitated before bottling. Otherwise, after a certain period of time, the veiled oil turns into a separate phase system and forms a brown residue that settles into the bottom of the container. It is usually considered unacceptable by consumers if it appears in bottles [2]. This deposit is made by solids as well as a certain amount of water, whose characteristics affect the settling time. The amount of material in suspension in the oil changes with the extraction system; with the traditional extraction system, it is about 8% [2], while in a continuous extraction plant (after the vertical centrifuge), the suspended solids were, are on average, equal to 0.01% [3]. The VOO turbidity affects color and appearance of olive oil [4].

In oil musts, the water content is highly variable with an average value of roughly 0.5% by weight [5], while the International Olive Oil Council suggests an upper limit of 0.2% [6]. The presence of water could reduce the rate of oxidation and improve the stability of VOO [7] because water is rich in polar compounds, especially phenols with a strong antioxidant effect [8].

Suspended solids are usually small olive fragments. They remain in the VOO after the extraction, and they consist of proteins, sugar glycosides, sugar bounded with proteins, phospholipids, and phenolic compounds [2, 3].

Some of the above-mentioned compounds have amphiphilic behavior showing both lipophilic and hydrophilic properties. They organize themselves in colloidal forms (mainly in reverse micelles and in lamellae because these forms are energetically preferred) and aggregate at the interphase between water and oil [9]. Colloidal suspended solids have a large reactive surface and, containing both water and oil, allow the presence of both lipophilic and hydrophilic compounds. The size of such colloidal formations has been studied by Papadimitriou et al. [5], who relate the average size of these suspensions with the extraction process. Suspensions give to the VOO a cloudy appearance and cause hydrolytic reactions, which could result in sensorial defects [5].

Furthermore, solids and water in cloudy VOO allow the development of a microflora, mainly represented by yeasts [10]. Before olive processing, microflora lives on the olive carposphere and migrates into the oil together with the olive fragments and water. The substances on the olive fragments and the water allow these micro-organisms to survive. In just a few hours after the olive oil extraction, some yeasts such as *Candida* and *Saccharomyces* are able to colonize the environment [11]. The selected microflora could survive during the entire storage period and could damage the sensory characteristics of VOO. It has been demonstrated that some lipase-producing yeasts can hydrolyze triglycerides triacylglycerols in different manners according to the size of suspended micro-drops of vegetation water [11, 12]. The presence of water and solids together with some micro-organisms could lead to the formation of the "muddy-sediments" and "rancid" defects after a short storage time [13].

Changes in chemical and sensorial parameters in oil musts have been recently described in the literature [14], while different strategies have been developed to eliminate part or the whole suspended solids fraction.

http://dx.doi.org/10.5772/64401

3. Vertical centrifugation

To produce an olive oil clearer and stable, suspended solids are removed with static or dynamic filtration. The vertical centrifuge represents the most widespread technology for the clarification of the olive oil must coming from the decanter. Almost every olive oil extraction plant, working with the continuous system is equipped with this device. The vertical centrifuge removes many of the present impurities due to the difference in specific weight between water and oil. The physical principle of operation is the reduction in the settling time obtained by replacing the gravitational acceleration with a centrifugal acceleration provided by the machine. The sedimentation rate with the gravitational acceleration is governed by Stokes' law:

$$V_c = d^2\left(sw_1 - sw_2\right) g / 18\eta \tag{1}$$

where V_c is the sedimentation rate, d is the equivalent diameter of the particles to be removed, sw_1–sw_2 is the difference in specific weight of the substances to be separated, g is the gravitational acceleration (9.81 m/s^2), and η is the viscosity of the main phase. In vertical centrifuge, the term g is replaced by the squared angular speed of rotation of the centrifuge (at n rpm), multiplied by the radius of the centrifuge. The Stokes equation for the calculation of the rate of sedimentation in the vertical centrifuge then becomes:

$$V_c = d^2 \left(sw_1 - sw_2\right) \left(2\pi / 60\right)2r / 18\eta \tag{2}$$

The vertical centrifuge usually works at rotational speed of approximately 6000–6500 rpm on its axis. The particles to be separated move perpendicularly to the plane of decantation and quickly settle on the wall of the vessel. Thus, the high rotational speed allows sedimentation up to 20,000 times more quickly than those achievable with the gravitational acceleration, providing obvious benefits in terms of operational capability. The centrifuge, after a short lag phase due to the filling of the working chamber with the liquid to be clarified, works continuously, and it is usually able, by means of the lowering of a shutter with hydraulic control, to "shoot" outside the separated sediment and water. The central body has a vertical axis rotor, which allows centrifugation of the process liquid. The rotor is provided with a central feed tube, which carries a series of vertical disks superimposed and fixed to one another at a distance of about 1 mm. Near to the axis of rotation, these disks are perforated to form vertical channels. The disks have different functions: they reduce the turbulence of the introduced process liquid in the drum, reduce the radial space that the solid particles must travel to reach the collection chamber, and keep separated the liquid from the solid particles. In this way, the sedimentation speed and clarification are improved.

The oil is continuously introduced through the axial feed tube placed on the top of the drum and accelerates up to the operating rotational speed. Then, the oil rises through the conical spaces of the stack of disks and separates, thanks to the effect of the centrifugal force. Afterward, a centripetal pump sends it to the outlet pipe. The centripetal pump is placed at the exit of the liquid in the upper part of the drum. The kinetic energy of the liquid having an impact on the vanes is converted into pressure energy (3–6 bar) and pushes the oil in the outlet line.

The main technical parameters of vertical centrifuges are:

- nominal flow: the maximum flow capacity (L/h) that the centrifuge can reach operating water;
- volume of the sludge chamber: storage volume (L) of the solid decanted;
- hourly capacity: quantity of product obtainable from a murky clarified. It can be estimated with the following report and is a function of the nominal flow rate and the volume of the sludge chamber:

$$Q = VP_n / t\Delta s \quad (3)$$

where Q is the hourly flow rate, V is the volume of the sludge chamber, P_n is the nominal flow rate, t is the interval between the "shoots," and Δs is the percentage of suspended solids separate;

- effective range: less than or equal to the hourly capacity. Lower if the power cuts out during the shooting and the same if this does not happen; and
- flow factor: it is calculated based on the number of revolutions of the drum, the number of disks, their diameter, and their angle of inclination [15].

Despite the operative benefits provided, vertical centrifuge has several negative effects on olive oil quality.

First of all, during the centrifugation, operation water is generally added to the VOO. This water reduces the concentration of phenolic compounds, because they are generally hydrophilic molecules, and consequently decreases the stability of the VOO [16, 17]. The amount of each compound transferred from the oily phase to the water phase is regulated by the partition coefficient between water and oil. This coefficient is a function of the solubility of each compound in the two phases. The partition coefficients between oil and water for some phenolic compounds were calculated by Rodis et al. [17]. They were found to be highly variable and ranging from 0.0004 of hydroxytyrosol to 0.187 dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol at 25°C. The exception is the elenolic acid linked to hydroxytyrosol that showed a value of 11.8 [17]. These values show how the added process water can be detrimental for the quality of olive oil.

In the vertical centrifuge, oxygen is dissolved in the VOO. The latter is almost saturated in oxygen, while the oil must from the decanter shows half of the saturation value. Dissolved oxygen is quickly consumed by the VOO; this has been correlated with a considerable shelf life reduction. A linear regression between dissolved oxygen and peroxide number has been found in olive oils [18]. In the same work, a shelf life test showed the effect of high initial

http://dx.doi.org/10.5772/64401

concentration of dissolved oxygen during the storage. In both cases, there is a linear increase in peroxide value during storage. However, the slopes of the two lines are significantly different, and the centrifuged oil reaches the legal limit (20 meqO_2/kg—EC2568/91) more quickly than the non-centrifuged oil. Therefore, it is reasonable to assume that the dissolved oxygen added from the vertical centrifuge initiates the reactions of autoxidation during storage. Other negative effects of vertical centrifuge are higher values of K_{232} and lower concentration of biophenols during the olive oil storage [18].

To avoid or to reduce the negative effects of vertical centrifugation, some technology has been proposed, for example, centrifugation under inert gas [19] and nitrogen stripping [20]. The blanketing of the vertical centrifuge with an inert gas protects the VOO from oxidation and provides benefits in term of oxidative indexes (i.e., peroxide number and K_{232}). Inert vertical centrifuges are used in oenology [21] but only few in the VOO industry due to their high purchase costs.

The nitrogen stripping technique removes the dissolved oxygen from VOO. Nitrogen stripping results in lower oxidation of VOO (i.e., lower peroxide number). However, after stripping, a loss of some VOO key odorants (i.e., E-2-hexenal) is observed [20]. Stripping appears to be promising for VOO, but other studies are required to deeply understand its effect on the product. Inert centrifuges and nitrogen stripping could be useful to protect VOO from oxidation, but they cannot prevent the losses of phenolic compounds due to the use of process water.

In recent years, some strategies to avoid vertical centrifuge have been implemented. Altieri and coworkers propose an online sedimentation approach [22]. Sedimentation is an ancient procedure used to clarify vegetable oils, requiring large basins and long times. The Altieri and co-workers' [22] approach reduces the time required by sedimentation and allows a fast VOO clarification.

Some small olive mills, producing high quality olive oils, start to filter the VOO directly from the decanter, avoiding the vertical centrifuge. This approach requires a careful sizing of the filter to equal the working capacity of the decanter. If the filter is well sized, this approach seems to be able to protect the VOO quality, avoiding the introduction of oxygen and water due to the vertical centrifugation [14, 23].

4. Filtration

Filtration is one of the most debatable steps in olive oil production. Some consumers appreciate veiled olive oils recently produced, while others prefer filtered olive oil.

Filtration could be considered one of the simpler and spread solid-liquid separation. During filtration, a suspension is forced into a porous medium able to hold the solids and let the liquid pass through. The liquid will be consequently clearer. Two theoretical models explain the filter behavior: surface and depth filtration. In the former, the porous medium has holes smaller than the suspended particles. In the latter, the separation is due to the adsorption of particles into the porous. In this case, the porosity is greater than the retained solids. The division into surface filters and depth is a simplification of what happens in real filters where, usually, both theoretical models occur simultaneously. For instance, the layer of solid particles that are deposited on a surface filter exerts increasingly greater depth of action as it increases in thickness [24, 25].

The speed of a filtration process is defined as the quantity of filtrate (V) processed per unit of time (θ). This speed is governed by the equation of Hagen-Poiseuille:

$$\frac{1}{A}\frac{dV}{d\theta} = \frac{\Delta P}{\mu\left(\alpha wV / A + r\right)} \tag{4}$$

where A is the surface of the filter, ΔP is the difference of pressures at the two sides of the filter medium, μ is the viscosity, α is a coefficient due to the filtered resistance that has accumulated in the medium, w is the content of solids in the filtrate in weight per unit of volume, and r is the resistance of the filter medium. From the equation, it can be noted that the driving force that allows the filtration of a solid-liquid mixture is the pressure difference between the two sides of the filter media (ΔP). The pressure difference is theoretically proportional to the speed with which the process takes place. Actually, the physicomechanical characteristics of the solid residue of olive oil divert from linearity, and the pressure increase is often not proportional to the increase in the rate of filtration. From the formula, it is easy to understand the importance of the correct size of the filter, hence the correct determination of the filtering surface (A). Another important parameter for VOO filtration is the temperature of the mixture during the process. Temperature does not appear directly in the equation of Hagen-Poiseuille but strongly influences the viscosity. The higher the temperature, the lower the viscosity of the oil to be filtered. For this reason, the filtration speed of VOO at 30°C is twice than a filtration at 15°C [26]. Finally, the parameter α produces strong variation during filtration. The parameter α is minimum at the beginning of the process and increases progressively until the filtration cycle ends due to the low speed. This effect is caused by the progressive increase in the amount of solids on the filter medium [27].

Many filtration technologies are adopted to remove solids and water from VOO: conventional systems such as filter tank, filter press [27], cross-flow systems [28], inert gas flow filtration systems, and filter bags, as reviewed by Frankel et al. [29].

The simplest filter used for VOO is the cotton filter (or "alla barese" filter, an old gravity filter that may be used in a small scale). The VOO is introduced into a vessel lying on the bottom of one or more layers of textile fiber. The filtered oil is collected in a second vessel placed at a lower level than the first. This type of filtration is carried out without pumps, using the gravity acceleration. The cotton filter is a cheap and easy solution. The main drawback of cotton filter is related to the very long time required for the operation. The pressure difference between the two parts of the filter is low due to the force of gravity and by the hydrostatic pressure of the column of oil. The filtering surface is limited. During this operation, the oil is exposed to oxygen and consequently to the risk of oxidation [27].

The most common filter used in small- and medium-sized production companies of VOO is the filter press. Filter press consists of a series of flat chambers placed vertically next to each other. The chambers are composed of a plate used for support and turbid oil distribution, and by the filter medium, composed of filter sheets. To avoid loss of oil when the pressure rises, chambers and filter sheets are adhered to each other through a central screw and two metal plates. The filter is dimensioned by choosing the appropriate number of filter media (sheets). They usually have a square shape and size between 20 and 120 cm [25]. The filter sheets for filter press are depth filters and consist mainly of cellulose [23]. To reduce the use of sheets and the VOO losses, the filter press could be coupled with stainless steel pre-filters [23].

On a larger scale, Kieselguhr filters are often used. In this kind of filters, the filtration adjuvant is continuously added to the olive oil suspension. That colloidal suspension gives rise to highly compressible deposits, and the adjuvant confers to the deposit stiffness and porosity to obtain good permeability and ensure higher flow throughout the filtration cycle. However, in the last years, the cross-flow filtration (membrane filtration) began to spread [28].

Membrane filtration is a very common practice for seed oils, which recently has also been proposed for the VOO. Membrane filters, when compared with other filtration systems, provide some benefits. For instance, they eliminate the filtration adjuvants. Usually, adjuvants retain VOO, causing some losses. Furthermore, the disposal of these adjuvants could be problematic. In the Kieselguhr filtered oils, it is common to find traces of some filter aids; this does not happen in the membrane-filtered oils. In addition to the solution of these problems, the membrane filtration allows to remove traces of heavy metals, such as copper, manganese, and iron, which sometimes contaminate the oils. The cross-flow filters are typically surface filters, characterized by a high efficiency of retention at low porosity [28].

Filtration affects chemical composition of VOO, changing the minor compound concentration and olive oil stability during storage [30]. Lower stability and greater susceptibility to alterations are attributed by some authors to cloudiness. However, water and suspended solid particles contain antioxidant compounds. Koidis and Boskou [3] explain this decrease in the stability of filtered oils on the basis of total phenol contents because some of these compounds are highly soluble in water. Filtration may cause a reduction in the stability of the oils, removing phenolic compounds [2, 30]. Furthermore, from the observations on a model system obtained by emulsifying the olive oil, Ambrosone and coworkers [7] concluded that the presence of dispersed water reduces the rate of oxidation. Hidalgo and co-workers [32, 33] pointed out that traces of peptide compounds may also play a stabilizing role. Observations related to a lower stability of filtered oils have been made by Tsimidou et al. [34] and Papadimitriou et al. [5].

Filtration affects not only the concentration of biophenols but also other minor compounds. Chlorophylls [28] and waxes [35] are reduced by this operation. On the other hand, tocopherols seem to be not affected by filtration [36, 37]. Finally, filtration can influence the aroma of the oils. Few studies focus on the effects of filtration on volatile organic compounds in olive oil. Bottino et al. [28] quantified losses ranging from 57 to 72% of carbonyl compounds (mainly aldehydes) with 5–13 carbon atoms. Bubola et al. [38] report similar changes and point out that ketones and compounds with five carbon atoms are more easily removed.

Brenes et al. [36] indicated that unfiltered oils have a higher peroxide value, K_{232}, and are prone to more rapid oxidation. These changes could be explained with the presence in the suspended solids of oxidative enzymes [39, 40], as well as esterases and glycosidases [36]. With water and solids removal, filtration could reduce the rate of enzyme activity, preserve the biophenol fraction, and retard the degradation. Filtration removes these enzymes and reduces the hydrolysis of secoiridoids [14]. Hence, in cloudy oils, phenolic alcohols, namely tyrosol and hydroxytyrosol, are quickly produced, while in filtered oil, the increase in phenolic alcohol concentrations is slower. This increase is due to the degradation of the secoiridoid fraction (oleuropein and ligstroside derivatives). These compounds remain quite stable in filtered oils over time. Regarding the volatile fraction, the same authors [14] found that compounds of LOX pathways such as E-2-hexenal and other C6 are constant in filtered oils. Molecules related to rancidity (i.e., 2,4-decadienal, 2,4-heptadienal, E-2-decenal, octane) and to a winey attribute (ethyl acetate) have been found to increase more quickly in cloudy than in filtered oils.

In conclusion, filtration provides greater flavor stability by reducing the formation rate of off-flavor compounds and the appearance of sensory defects. The absence of these defects is required by the European law to label olive oils as extra-virgin.

Author details

Lorenzo Guerrini and Alessandro Parenti*

*Address all correspondence to: alessandro.parenti@unifi.it

Department of Agricultural, Food Forestry Systems—Biosystems Engineering Section, Florence, Italy

References

[1] Morelló, J. R., Motilva, M. J., Tovar, M. J., & Romero, M. P. Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. Food Chemistry. 2004; 85(3):357–364.

[2] Lerker, G., Frega, N., Bocci, F., & Servidio, G. "Veiled" extra-virgin olive oils: dispersion response related to oil quality. Journal of American Oil Chemists' Society. 1994; 71(6): 657–658.

[3] Koidis, A., & Boskou, D. The contents of proteins and phospholipids in cloudy (veiled) virgin olive oils. European Journal of Lipid Science and Technology. 2006; 108(4):323–328.

[4] Gordillo, B., Ciaccheri, L., Mignani, A. G., Gonzalez-Miret, M. L., & Heredia, F. J. Influence of turbidity grade on color and appearance of virgin olive oil. Journal of the American Oil Chemists' Society. 2011; 88(9):1317–1327.

[5] Papadimitriou, V., Dulle, M., Wachter, W., Sotiroudis, T. G., Glatter, O., & Xenakis, A. Structure and dynamics of veiled virgin olive oil: influence of production conditions and relation to its antioxidant capacity. Food Biophysics. 2013; 8(2):112–121.

[6] International Olive Oil Council. Trade Standard Applying to Olive Oil and Olive Pomace Oils. 2009, COI/T.15/NC No3. Rev 8, 2015.

[7] Ambrosone, L., Angelico, R., Cinelli, G., Di Lorenzo, V., & Ceglie, A. The role of water in the oxidation process of extra virgin olive oils. Journal of the American Oil Chemists' Society. 2002; 79(6):577–582.

[8] Bakhouche, A., Lozano-Sánchez, J., Ballus, C. A., Martínez-García, M., Velasco, M. G., Govantes, Á. O., Gallina-Toschi, T., Fernández-Gutiérrez, A., & Segura-Carretero, A. Monitoring the moisture reduction and status of bioactive compounds in extra-virgin olive oil over the industrial filtration process. Food Control. 2014; 40:292–299.

[9] Xenakis, A., Papadimitriou, V., & Sotiroudis, T. G. Colloidal structures in natural oils. Current Opinion in Colloid & Interface Science. 2010; 15(1):55–60.

[10] Ciafardini, G., & Zullo, B. A. Microbiological activity in stored olive oil. International Journal of Food Microbiology. 2002; 75(1):111–118.

[11] Ciafardini, G., Zullo, B. A., Cioccia, G., & Iride, A. Lipolytic activity of Williopsis californica and Saccharomyces cerevisiae in extra virgin olive oil. International Journal of Food Microbiology. 2006; 107(1):27–32.

[12] Ciafardini, G., Zullo, B. A., & Iride, A. Lipase production by yeasts from extra virgin olive oil. Food Microbiology. 2006; 23(1):60–67.

[13] Zullo, B. A., Cioccia, G., & Ciafardini, G. Effects of some oil-born yeasts on the sensory characteristics of Italian virgin olive oil during its storage. Food Microbiology. 2013; 36(1):70–78.

[14] Fortini, M., Migliorini, M., Cherubini, C., Cecchi, L., Guerrini, L., Masella, P., & Parenti, A. Shelf life and quality of olive oil filtered without vertical centrifugation. European Journal of Lipid Science and Technology. 2015; 117. In press DOI: 10.1002/ejlt.201500229.

[15] De Vita, P. Corso di meccanica enologica. Hoepli, Milano, Italy. 1997. 532 p.

[16] Di Giovacchino, L., Solinas, M., & Miccoli, M. Effect of extraction systems on the quality of virgin olive oil. Journal of American Oil Chemists' Society. 1994; 71(11):1189–1194.

[17] Rodis, P. S., Karathanos, V. T., & Mantzavinou, A. Partitioning of olive oil antioxidants between oil and water phases. Journal of Agricultural and Food Chemistry. 2002; 50(3): 596–601.

[18] Masella, P., Parenti, A., Spugnoli, P., & Calamai, L. Influence of vertical centrifugation on extra virgin olive oil quality. Journal of the American Oil Chemists' Society. 2009; 86(11):1137–1140.

[19] Masella, P., Parenti, A., Spugnoli, P., & Calamai, L. Vertical centrifugation of virgin olive oil under inert gas. European Journal of Lipid Science and Technology. 2012; 114(9): 1094–1096.

[20] Masella, P., Parenti, A., Spugnoli, P., & Calamai, L. Nitrogen stripping to remove dissolved oxygen from extra virgin olive oil. European Journal of Lipid Science and Technology. 2010; 112(12):1389–1392.

[21] Jackson, R. S. Wine science: principles and applications. Academic Press, San Diego. 2008.

[22] Altieri, G., Di Renzo, G. C., Genovese, F., Tauriello, A., D'Auria, M., Racioppi, R., & Viggiani, L. Olive oil quality improvement using a natural sedimentation plant at industrial scale. Biosystems Engineering. 2014;122:99–114.

[23] Guerrini, L., Masella, P., Migliorini, M., Cherubini, C., & Parenti, A. Addition of a steel pre-filter to improve plate filter-press performance in olive oil filtration. Journal of Food Engineering. 2015; 157:84–87.

[24] Peri, C. La filtrazione nelle industrie alimentari. Edizioni AEB, Brescia, Italia. 1983. 478 p.

[25] Sutherland, K. Filters and filtration handbook. 5th ed. Elsevier, UK. 2008. 523 p.

[26] Bonnet, J. P., Devesvre, L., Artaud, J., & Moulin, P. Dynamic viscosity of olive oil as a function of composition and temperature: a first approach. European Journal of Lipid Science and Technology. 2011; 113(8):1019–1025.

[27] Peri, C. The extra-virgin olive oil handbook. Wiley Blackwell, United Kingdom. 2014. 280 p.

[28] Bottino, A., Capannelli, G., Comite, A., Ferrari, F., Marotta, F., Mattei, A., & Turchini, A. Application of membrane processes for the filtration of extra virgin olive oil. Journal of Food Engineering. 2004; 65(2):303–309.

[29] Frankel, E., Bakhouche, A., Lozano-Sánchez, J., Segura-Carretero, A., & Fernández-Gutiérrez, A. Literature review on production process to obtain extra virgin olive oil enriched in bioactive compounds. Potential use of byproducts as alternative sources of polyphenols. Journal of Agricultural and Food Chemistry. 2013; 61(22):5179–5188.

[30] Lozano-Sánchez, J., Cerretani, L., Bendini, A., Segura-Carretero, A., & Fernández-Gutiérrez, A. Filtration process of extra virgin olive oil: effect on minor components, oxidative stability and sensorial and physicochemical characteristics. Trends in Food Science & Technology. 2010; 21(4):201–211.

[31] Frega, N., Mozzon, M., & Lercker, G. Effects of free fatty acids on oxidative stability of vegetable oil. Journal of the American Oil Chemists' Society. 1999; 76(3):325–329.

[32] Hidalgo, F. J., Alaiz, M., & Zamora, R. Determination of peptides and proteins in fats and oils. Analytical Chemistry. 2001: 73(3):698–702.

[33] Hidalgo, F. J., Alaiz, M., & Zamora, R. Low molecular weight polypeptides in virgin and refined olive oils. Journal of the American Oil Chemists' Society. 2002; 79(7):685–689.

[34] Tsimidou, M. Z., Georgiou, A., Koidis, A., & Boskou, D. Loss of stability of "veiled" (cloudy) virgin olive oils in storage. Food Chemistry. 2005; 93(3):377–383.

[35] Parenti, A., Spugnoli, P., Baldi, F., Masella, P., Calamai, L., & Mattei, A. Preliminary observations on veiled olive oil turbidity with regards to wax content. La Rivista Italiana Delle Sostanze Grasse. 2008; 85:221–228.

[36] Brenes, M., García, A., García, P., & Garrido, A. Acid hydrolysis of secoiridoid aglycons during storage of virgin olive oil. Journal of Agricultural and Food Chemistry. 2001; 49(11):5609–5614.

[37] Fregapane, G., Lavelli, V., León, S., Kapuralin, J., & Desamparados Salvador, M. Effect of filtration on virgin olive oil stability during storage. European Journal of Lipid Science and Technology. 2006; 108(2):134–142.

[38] Brkić Bubola, K., Koprivnjak, O., & Sladonja, B. Influence of filtration on volatile compounds and sensory profile of virgin olive oils. Food Chemistry. 2012; 132(1):98–103.

[39] Georgalaki, M. D., Sotiroudis, T. G., & Xenakis, A. The presence of oxidizing enzyme activities in virgin olive oil. Journal of the American Oil Chemists' Society. 1998; 75(2): 155–159.

[40] Valgimigli, L., Sanjust, E., Curreli, N., Rinaldi, A., Pedulli, G. F., & Rescigno, A. Photometric assay for polyphenol oxidase activity in olives, olive pastes, and virgin olive oils. Journal of the American Oil Chemists' Society. 2001; 78(12):1245–1248.

IntechOpen