Olive oil processing technologies and investments

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Arcas N. (ed.), Arroyo López F.N. (ed.), Caballero J. (ed.), D'Andria R. (ed.), Fernández M. (ed.), Fernandez Escobar R. (ed.), Garrido A. (ed.), López-Miranda J. (ed.), Msallem M. (ed.), Parras M. (ed.), Rallo L. (ed.), Zanoli R. (ed.).
Present and future of the Mediterranean olive sector

Zaragoza: CIHEAM / IOC
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 106

2013
pages 55-66

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Abstract. The commercial quality of Extra virgin olive oil (EVOO) is defined by the European Legislation (EC 61/2011), International Olive Council (IOC) and the *Codex Alimentarius*; it includes parameters describing the alteration state and assures the oil genuineness, but does not take into account the markers related to the sensory and healthy quality of the product. These properties of EVOO are strongly related to the amount of monounsaturated fatty acids and phenolic compounds (which, in particular, act as natural antioxidants and may contribute to the prevention of several human diseases) and volatile compounds. The main antioxidants are the lipophilic and hydrophilic phenolic compounds. While lipophilic phenols can be found in vegetable oils from other species, the hydrophilic phenols, such as secoiridoids, are exclusive of oils from *Olea europaea*. Moreover, they give bittern and pungent EVOO sensory notes. The volatile compounds responsible for EVOO flavour are due to the lipoxygenase pathway (LOX) that catalyses the genesis of C_5 and C_6 saturated and unsaturated aldehydes, alcohols and esters. These compounds are correlated to the "cut grass" and "floral" sensory notes of EVOO. The concentration of fatty acids, phenols and volatile compounds is largely affected by agronomic factors and by oil extraction conditions during crushing, malaxation and EVOO separation. The malaxation conditions such as temperature and oxygen concentration of paste during process regulate the activities endogenous enzymes polyphenoloxidase (PPO), peroxidase (POD) and lipoxygenase (LOX), activated during crashing with a strong effect in the final concentration of hydrophilic phenols and volatile composition of EVOO. Moreover, new EVOO extraction technologies are also oriented towards the valorisation of their by-products.

Keywords. Extra virgin olive oil quality – Phenols – Volatile compounds – Technological conditions – Endogenous enzymes.

Processus technologiques et investissements pour l'huile d'olive

Résumé. *La qualité commerciale des huiles d'olive vierges extra (HOVE) est définie par la législation du secteur, elle inclut des paramètres décrivant l'état d'altération et assure de l'authenticité des huiles, mais ne prend pas en considération les marqueurs liés à la qualité sensorielle et salutaire du produit. Ces propriétés de l'HOVE sont liées à la quantité d'acides gras monoinsaturés, de composés phénoliques et de composés volatils. Les principaux antioxydants sont des composés phénoliques lipophiles et hydrophiles. Tandis que les phénols lipophiles peuvent être présents dans les huiles végétales, les phénols hydrophiles, tels que les sécoïridoïdes, dans l'HOVE sont exclusifs d'*Olea europaea*. En outre, ils donnent à l'HOVE des notes sensorielles amères et piquantes. Les composés volatils responsables de l'arôme de l'HOVE sont dus à la voie de la lipoxygénase (LOX) qui catalyse la genèse des aldéhydes, des alcools et des esters en C_5 et C_6 saturés et insaturés. Ces composés sont corrélés aux notes sensorielles d'"herbe coupée" et "florales" de l'HOVE. La concentration en acides gras, en phénols et en composés volatils est influencée par les facteurs agronomiques et par les conditions d'extraction de l'huile au cours du broyage, du malaxage et de la séparation de l'HOVE. Les conditions de malaxage telles que la température et la concentration en oxygène de la pâte pendant le processus peuvent réguler l'activité des enzymes endogènes polyphénoloxydase (PPO), peroxydase (POD) et lipoxygénase (LOX), avec un fort effet sur la concentration finale en phénols hydrophiles et la composition volatile de l'HOVE. En plus, les nouvelles technologies d'extraction de l'HOVE sont aussi orientées vers la valorisation de leurs sous-produits.*

Mots-clés. *Qualité des huiles d'olive vierges extra – Phénols – Composés volatils – Conditions technologiques – Enzymes endogènes.*

I – Introduction

The International Oil Council (IOC, 2010) and the European Community (EU Reg. 61/2011) establish the characteristics of different olive oils and define quality and authenticity criteria for a correct olive oil commercial classification. They have defined the marketable quality of virgin olive oil by dividing it into three different commercial categories [extra virgin (EVOO), virgin and lampante] on the basis of some analytical parameters evaluating the hydrolytic alteration (such as the free acidity) and the oxidation state [such as the peroxide value and the UV specific extinction coefficients (K232 and K270)]. On the other hand, in order to guarantee the genuineness of oil they take into account other analytical markers, such as waxes, sterols, aliphatic and triterpenic alcohols trans-isomers of fatty acids, fatty acids and triacylglycerols composition, stigmastadiens, etc. The sensory analysis integrates the analytical definition of the EVOO. This approach has been proposed by the International Olive Council (IOC, 1987) and accepted by the European Community (EC, 1989/2003). The first aim of this analysis is the control of the off-flavours' occurrence which are not admitted in the EVOO category. These sensory "defects" have been well defined by the International Olive Council (IOC) that also standardizes the procedure for the evaluation of the sensory *flavour* and *off-flavours* (IOC and Reg. EU 1989/03).

However, the current olive oil official regulations do not take into account analytical markers, like natural antioxidant compounds and monounsaturated oleic acid and squalene concentrations, for determining healthy and sensory properties of the EVOO although they are directly involved in conferring benefits to human health. In this respect, the current EVOO class does not mention any information concerning the above discussed parameters on the label and, therefore, it is not able to inform the consumer about the health properties of the product. In recent years, more and more attention has been given to a superior concept of EVOO quality that is based on the sensory, nutritional and healthy properties of this product. These aspects are due to its high content of monounsaturated oleic acid, squalene and natural antioxidant such as phenolic compounds, tocopherols and carotenoids (López-Miranda *et al.,* 2010; Bach-Faig *et al.,* 2011; Cicerale *et al.,* 2011), while the sensory properties (mainly aroma) of EVOO is the result of a complex mixture of volatile compounds, C_5 and C_6 saturated and unsaturated aldehydes, alcohols and esters responsible for some typical *flavour*, such as "cut grass", "haylike" and "floral", and also of hydrophilic phenols for bitter and pungent notes (Angerosa *et al.*, 2004; Servili *et al*., 2004, 2009a). Moreover, these substances show a high antioxidant activity and play an important role in the prevention and/or reduction of chronic degenerative events based on inflammatory processes and chronic-degenerative diseases such as cardiovascular-cerebral diseases (EFSA, NDA, 2011) and cancer (Servili *et al.,* 2009a; Obied *et al.,* 2012).

The nutritional importance of EVOO is mainly attributed to its high content of monounsaturated fatty acids (oleic acid, in particular), but in the last decade it has been observed a significant variability in the EVOO oleic acid content, whose range traditionally was fixed between 54% and 82% of the overall fatty acids quantity. This strong variability is strictly related to the extensions of the olive growing in several new areas where the produced oils show a poor content of oleic acid, lower than 50%. It is a matter of fact that this aspect is in contrast with the health values of EVOO (Terés *et al*., 2008; Lopez-Huertas, 2010).

Moreover, the same variability has been assessed for EVOO tocopherols and hydophilic phenols. In particular, α-tocopherol is the main tocopherol evaluated and it shows a great variability in the EVOO marketable class. In fact, it has been found that its value ranged between 23 and 730 mg/kg on 430 analyzed samples. The same remarks can be made for hydrophilic phenols. The EVOO polyphenols represent a group of secondary plant metabolites not often present in other oils and fats. The hydrophilic phenols class is the most important one and includes phenolic alcohols and acids, flavonoids, lignans and secoiridoids (Servili *et al.,* 2004; Obied *et al.,* 2008): the latter, exclusively present in the Oleacease family plants of which the olive is the only edible fruit, are the most important fraction under a biological point of view.

Secoiridoids are in fact the most abundant polyphenols in EVOO. They are represented by the dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA-EDA or *p*-HPEA-EDA), an isomer of oleuropein aglycone (3,4- DHPEA-EA) and the ligstroside aglycone (*p*-HPEA-EA) (De Marco *et al.,* 2007; Obied *et al.,* 2007, 2008; Servili *et al.*, 1999; 2004; 2009a). As discussed above with respect to the α-tocopherol, in EVOO the range of concentration variability of these compounds is very large. In fact, more than 500 EVOO samples were evaluated and the obtained results showed a variability between 50 and 900 mg/Kg. Therefore, it would be of fundamental importance to redefine the relationship between the EVOO marketable classification and its true quality.

The process innovation in the field of EVOO should follow a new approach towards the oil quality, which is strongly related to the content of phenolic and volatile compounds (which are the main responsible for the positive aroma). The qualitative and quantitative composition of both the volatile and the phenolic fractions is affected by several agronomic factors (such as cultivar, ripening stage, geographic and genetic origin of olive fruit, olive trees irrigation) as well as by technological aspects (Angerosa *et al.*, 2004; Servili *et al.,* 2004; 2009b; Inglese *et al*., 2011). In this respect, this new approach should take into account the effects of the temperature and of the oxygen control during the malaxation on phenolic and volatile compounds to improve the quality of the oil, thanks to the control on the enzymatic activities involved in the release and oxidation of phenols and in the biogenesis of flavor. In this context, the new challenge to the competiveness improvement of the virgin olive oil production has the following goals: (i) the optimization of the operative conditions to improve the virgin olive oil healthy and sensory properties; and (ii) the by-products' valorisation (stoned olive pomaces and vegetation waters).

II – EVOO mechanical extraction system and quality

The volatile and phenolic composition of virgin olive oil is strongly affected by agronomic factors (the genetic and geographical origin of the fruits) as well as by the enzymatic reactions such as polyphenoloxidase (PPO), peroxidase (POD) and lipoxygenase (LOX), occurring during the different phases of the mechanical extraction process of the oil (Di Giovacchino *et al.,* 1994; Caponio *et al.*, 1999; Servili *et al*., 2004). Some endogenous enzymes, crushing activated, play an important role to determine the amount of phenolic and volatile compounds in EVOO. After crushing, all endogenous enzymes are still active. The PPO and POD, catalyzing the oxidation of phenols, reduce their concentration in pastes and oils, and LOX, through a cascade pathway, produce of the C_5 and C_6 saturated and unsaturated aldehydes, alcohols and esters responsible for aromatic notes of "cut grass" and "floral" oils virgin olive oil.

The crushing method has a clear influence on the phenol concentration and volatile composition of EVOO. In fact, the phenolic compounds are most concentrated in the pulp, while little amounts of these substances are content in stone and seed (Servili *et al.*, 1999). The use of a hammer with a differentiated effect on the constitutive parts of the drupes, such as blade crusher, teeth crusher, pre-crusher or stoning crushing, reduces the seed tissues degradation, limiting the release of POD in the pastes, improves the concentration hydrophilic phenols in the EVOO preventing their oxidation during malaxation (Fig. 1) (Servili *et al*., 2007a). Moreover, the crushing system also affect the volatile composition of EVOO. The use of a hammer mill crusher as well as other crushers which determine a more violent grinding of pulp tissues, causes an increase of the olive paste temperature and a reduction of HPL activity (Servili *et al.*, 2002; Angerosa *et al.*, 2004). The olive stoning during EVOO mechanical extraction process increases the phenolic concentration in EVOO (Angerosa *et al.,* 1999; Lavelli and Bondesan, 2005; Mulinacci *et al*., 2005; Amirante *et al.*, 2006; Servili *et al.,* 2007a) and, at the same time, modifies the composition of volatile compounds produced by the LOX pathway, increasing the concentration of those volatile substances correlated to the "green" sensory notes (Angerosa, 2004; Servili *et al.,* 2007a).

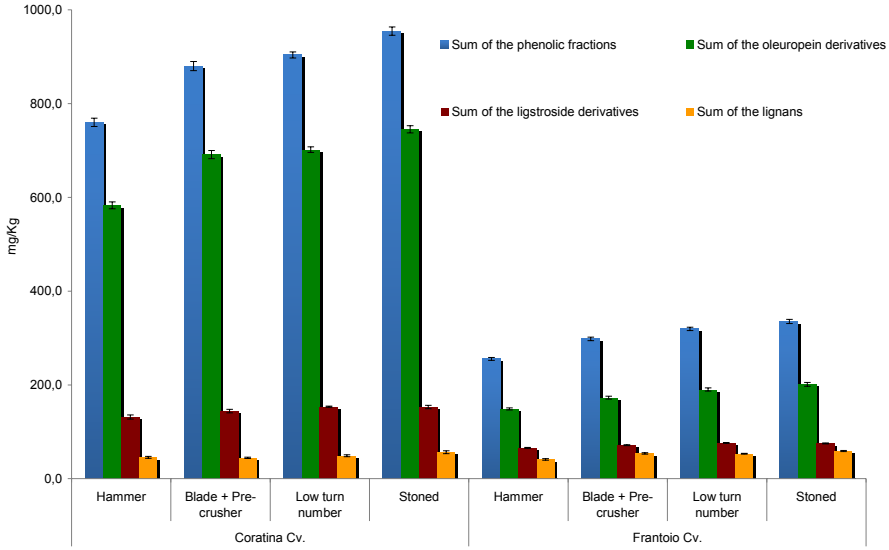

Fig. 1. Phenolic composition (mg/kg) of EVOOs (*Coratina* and *Frantoio* Cvs.) obtained by different crushing methods. Results are mean value of three indipendent determinations ± standard deviation (Servili *et al.*, 2007a).

The aim of the malaxation is to break up of oil/water emulsion and to assure the aggregation of the dispersed oil droplets in the paste to facilitate the subsequent process of the oil separation. In recent years the role of the operative conditions applied during malaxation that largely affect EVOO quality has been deeply investigated. In fact, the concentrations of the phenolic and volatile compounds are strongly related to the management of three main operating variables: temperature, time and availability of oxygen in the malaxer head-space. During the malaxation the LOX activity, representing the basis for the oil flavour production, should be supported; on the contrary the activities degrading phenolic compounds, of which the PPO and POD are responsible, should be inhibited. At this regards the decrease of O_2 inhibits the POD and PPO activities, increasing the amount of hydrophilic phenols in the olive pastes and in the corresponding EVOOs (Fig. 2). Furthermore the natural release of CO_2 due to the olive cell metabolism during malaxation, reduces the O_2 contact with the paste in this phase (Parenti *et al*., 2006 a; 2006b; Servili *et al*., 2008a).

The influence of the malaxation temperature on the concentration of phenolic compounds in EVOO has recently been the object of new investigations (Boselli *et al*., 2009; Gómez-Rico *et al*., 2009). The relationships between temperature and phenolic concentration are also affect by the low amount of O_2 occurring in the covered malaxer. Low O_2 concentration in the malaxed pastes inhibits phenolic oxidative degradation performed by PPO and POD activity and the temperature increase improves the phenolic solubility in the EVOO (Servili *et al*., 2008a; 2008b; Taticchi *et al.*, 2013).

In a recent work the effect of temperature and oxygen level during malaxation on the pastes and the related EVOOs extracted from four Italian cultivars was evaluate. The results showed that the occurrence of phenols in the pastes and the oils is strongly dependent from the temperature of malaxation. All oils from the analyzed *cvs*. are showing direct dependence between temperature and increase of phenolic concentration during malaxation with the highest value at 35°C. The amount of secoiridoids expressed as the sum of the phenolic fractions and oleuropein and ligstroside derivatives is increased increasing the temperature of the process, differently the concentration of lignans does not show any influence by the temperature (Fig. 3).

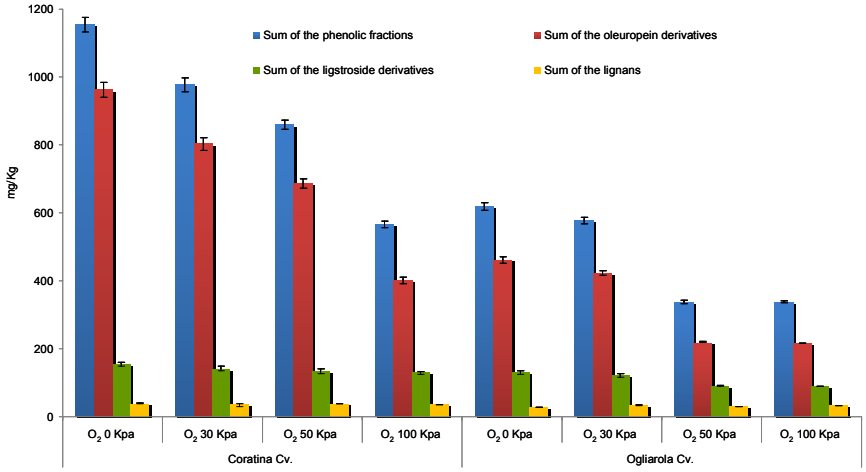

Fig. 2. Phenolic composition expressed as sum of HPLC phenolic fractions of the EVOOs (*Coratina* and *Ogliarola* Cvs.) malaxed at different O_2 concentrations. The data are the mean values of two independent experiments analysed in duplicate (Servili *et al*., 2008a).

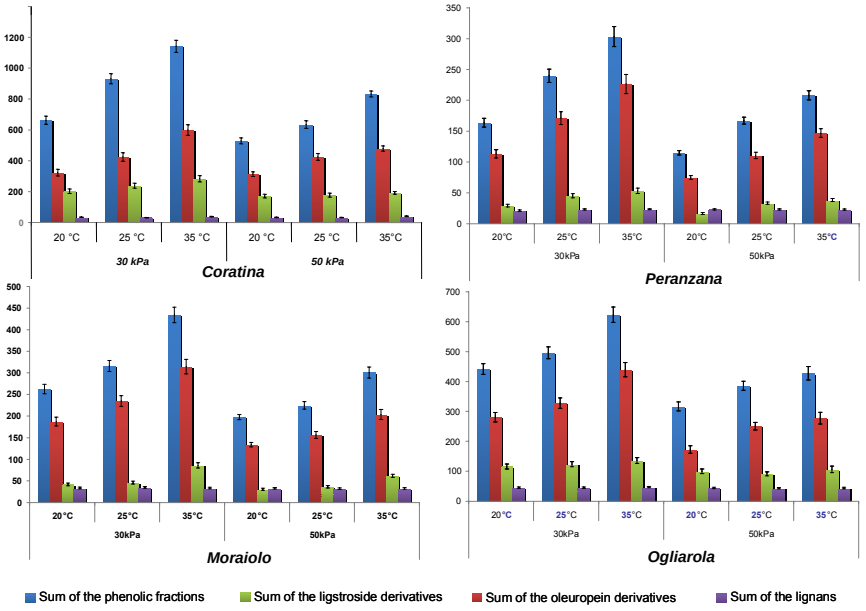

Fig. 3. Phenolic composition (mg/kg) of EVOOs obtained malaxing at different temperatures and O_2 levels. The data are the mean values of two independent experiments analysed in duplicate (Taticchi *et al*., 2013).

In addition the increase of the phenolic concentration due to the temperature is significantly related to the cv within a variability range of 85%-41% and the higher values are for the *Peranzana* and *Coratina* cvs at working temperature from 20°C to 35°C. The same Figures 2-3

show that, at constant temperature of the process, the oils of all cultivars malaxed with 50 kPa of O_2 are characterized by a phenolic concentration lower than the sample extract with 30 kPa of O_2 in the headspace of the kneader. This data confirms that the oxygen actively participates in the decrease of the phenolic concentration, above atmospheric values (30 kPa), increasing the processes of oxidative degradation. The data indicate the oxygen is actively involved in the reduction of the phenolic concentration above atmospheric values (30 kPa) increasing oxidative process degradation (Servili *et al*., 2008a).

The temperature of the process seems to have a fundamental role in the regulation of the oxidative activities on phenolic compounds catalyzed by oxidoreductases (POD, PPO) (Fig. 4). In particular the low thermal stability observed for PPO at temperature higher than 35°C seems to be responsible of the increased amount of phenolic compounds due to its partial inactivation during the malaxation process (Fig. 5) (Taticchi *et al*., 2013).

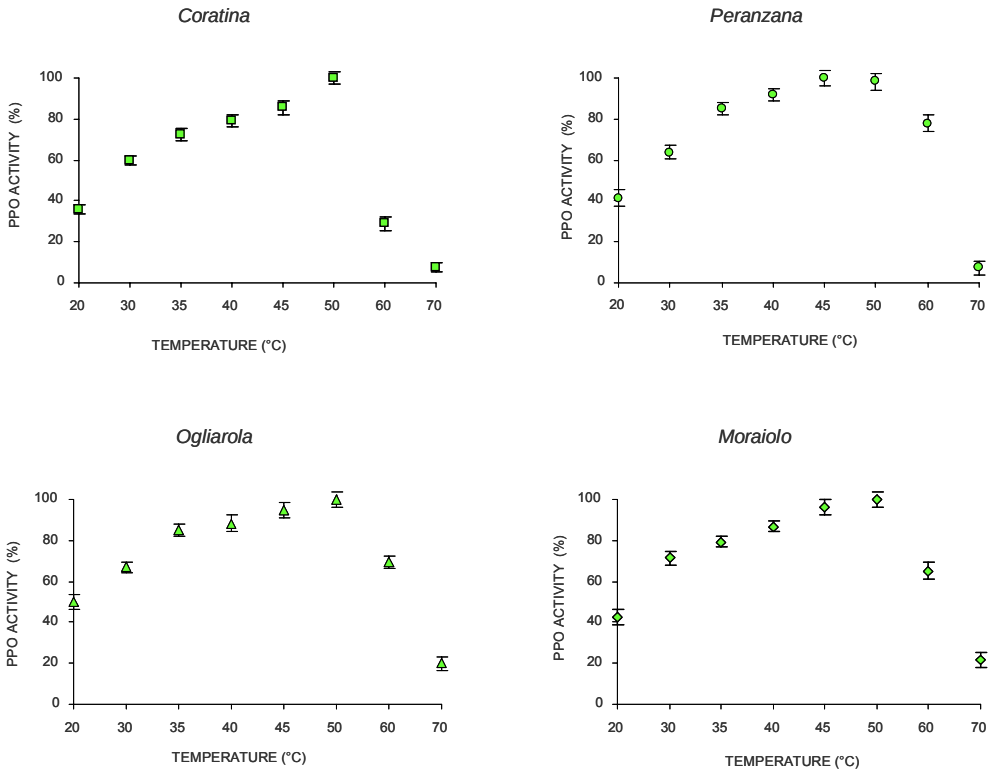

Fig. 4. Activity of the olive Polyphenoloxidase (PPO) at different temperatures. Data are the mean values of two independent experiments analysed in duplicate (Taticchi *et al*., 2013).

Furthermore volatile compounds in oils (Table 1) show significant quantitative differences according to the temperature of the malaxation and also to the *cvs*. Thus as regard to the aldehydes the highest concentration occurs at 25°C, with more significant differences for the *Coratina* and *Ogliarola* compared to the *Peranzana* and *Moraiolo*. As regard to the alcohols of the cvs *Moraiolo* and *Coratina* a trend of increasing concentration as a function of temperature increase was observed, while for *Peranzana* and *Ogliarola* significant variations were showed. The concentration of esters seems to be more influenced by the processing temperature: the minor amounts are observed in all cases at 35°C while the effect of the malaxation temperature

of 20°C or 25°C is variable, since not a constant trend for the different cultivars of olives has been observed.

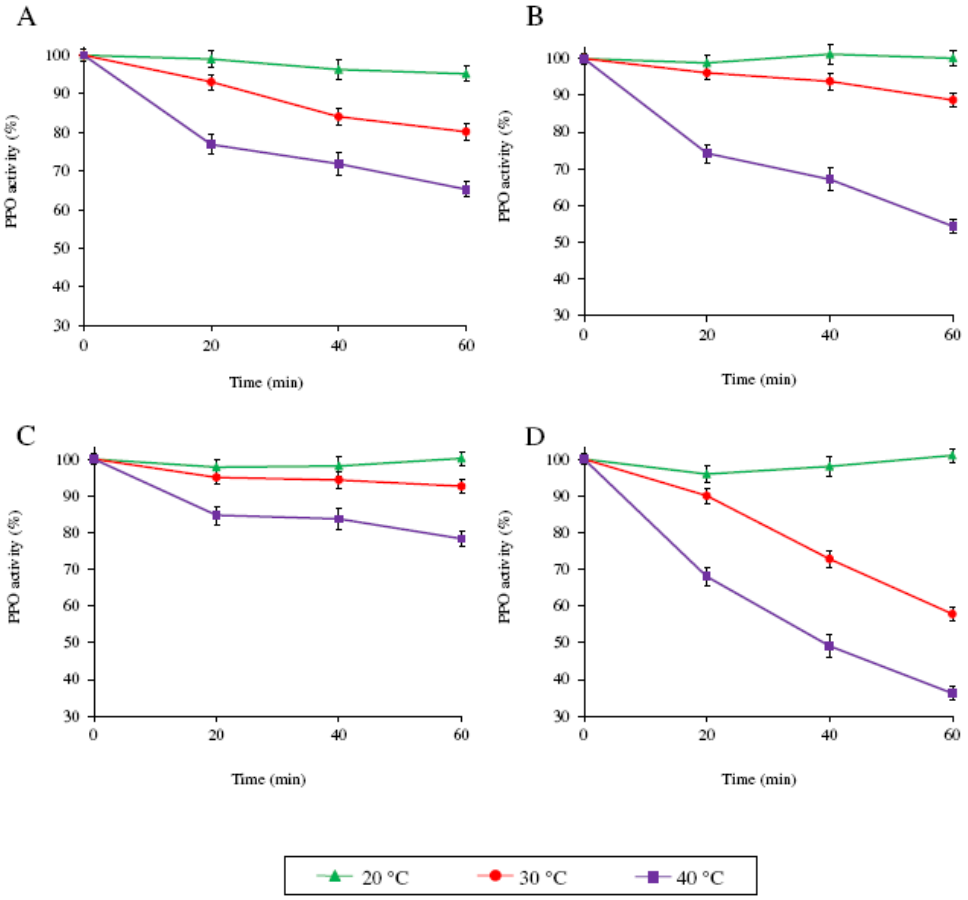

Fig. 5. Thermal stability after different times of incubation of PPO from olives studied at 20°C, 30°C and 40 °C on 4-methyl-catechol in different olive cultivars. A: *Moraiolo* cv.; B: *Coratina* cv.; C: *Peranzana* cv.; D: *Ogliarola* cv. The data are the mean values of two independent experiments analysed in duplicate (Taticchi *et al*., 2013).

Extraction system, pressure and centrifugation play an important role particularly in the phenolic composition of oil. In fact in the traditional centrifugation system, a large amount of water was added, before centrifugation, to reduce viscosity of pastes and improve oil yield. The water addition however, strongly reduce the phenolic concentration of oil and consequently modify its sensory and nutritional characteristics. In the last twenty years an evolution of this extraction system was performed to obtain a reduction of water addition during oil extraction. Due to this aspect the centrifuges can be classified in three groups: (a) the traditional tree phase centrifuges characterized by a water addition between 0.5 and 1 m^3 per ton; (b) the new tree phases centrifuges maximum level of water addition 0.2 and 1 m^3 per ton; and (c) two phases centrifuges that can work without water addition and did not produce vegetation waters as by-product of oil extraction process.

The oils extracted using the new systems showed a high phenolic concentration in comparison to that of the traditional centrifugation process because they reduced the loss of the hydrophilic phenols in the vegetation waters. In conclusion, the reduction of water addition before centrifugation and the temperature control during malaxation are the most important critical points of the oil extraction technology that strongly affect the EVOO quality. In addition the use of two phases centrifuges produced only pomaces, as secondary product, that can be used to produce organic compost. The organic compost may be important to improve fertility of soils particularly in warm area that are very common.

Table 1. Volatile composition (µg/kg) of EVOOs obtained by malaxing at different temperatures (Servili *et al*., 2008a). Aldehydes (Pentanal, Exanal, (E) 2-Pentenal, (E) 2-Exanal); Alcohols (1-Penten-3-ol, 1-Pentanol, (E) 2-Penten-1-ol, (Z) 2-Penten-1-ol, 1-Hexanol, (E) 3-Hexen-1-ol, (Z) 3-Hexen-1-ol, (E) 2-Exen-ol); Ester (Hexyl acetate, 3-Hexenyl acetate)

	20°C	25°C	35°C
	Moraiolo		
Aldehydes	11961.0 ± 694.4	17074.5 ± 995.0	16592.0 ± 972.4
Alcohols	1516.5 ± 43.3	1935.9 ± 61.9	2484.2 ± 88.9
Esters	128.0 ± 7.3	77.5 ± 4.4	24.0 ± 1.2
	Peranzana		
Aldehydes	19084.4 ± 1090.3	23408.0 ± 1330.7	18417.3 ± 1057.7
Alcohols	2398.7 ± 86.6	2462.2 ± 76.9	1911.0 ± 62.1
Esters	1247.7 ± 68.0	1198.0 ± 56.9	1316.2 ± 70.0
	Coratina		
Aldehydes	20139.0 ± 1184.2	34303.6 ± 2020.0	25678.4 ± 1509.5
Alcohols	1853.0 ± 72.5	3078.1 ± 122.0	4199.1 ± 169.3
Esters	75.5 ± 3.3	12.3 ± 0.5	0.0 ± 0.0
	Ogliarola		
Aldehydes	30713.0 ± 1807.1	38362.2 ± 2255.8	31144.7 ± 1825.7
Alcohols	3741.2 ± 154.8	3565.5 ± 130.9	3677.2 ± 145.9
Esters	29.5 ± 1.3	51.4 ± 2.3	9.8 ± 0.6

The volatile content is the mean value of three independent experiments ± standard deviation.

III – New approach to use of the EVOO by-products

The disposal of the by-products such as pomaces and olive vegetation waters (OVW) is considered an additional cost in the EVOO extraction process. The new approach to the EVOO processing should be oriented also towards the valorization of those wastes to improve the process profitability. The potential innovative use of EVOO by-products is related to their richness in hydrophilic phenols. The concentration of those compounds is largely affected by agronomic and technological conditions of VOO production. After the crushing and malaxation only a low portion of phenols, ranging between 1% and 3% of the overall phenolic concentration of olive fruit is released in the EVOO, while a larger amount occurs in the pomaces and OVW (Servili *et al.*, 1999; 2004; 2007a; 2007b; 2011a). The three phases extraction system, the most diffused in Italy, requires a dilution of the malaxed pastes with water (0.2 to 0.5 m^3/t of olives) producing 50-90 L of OVW/100 kg of olive pastes and 50-60 kg of olive pomaces/100 kg of olive pastes. The two phases system is at present largely used in Spain and it is characterized by a strong reduction of water consumption during the extraction process producing 70 kg of olive pomaces/100 kg of olive pastes.

The traditional destination of the pomaces is the extraction by organic solvents of residual oil to obtain crude pomaces oil. Some interesting opportunities for the pomaces valorization include the use as combustible to produce thermal energy from a renewable source, the compost production and the use as supplement in the animal feeding (Pauselli *et al*., 2007; Servili *et al*., 2007a).

The OVW valorization can be dependent from the recovery of the bioactive phenols contained in high amount in this by-product. The OVW consists of an emulsion composed by water, oil, mucillage and pectins and characterized by 3-16% of organic substances containing 1-8% of sugars, 1,2-2.4% of nitrogen compounds and 0.34-1.13% of phenolic compounds (Naionakis and Halvadakis, 2004). The secoiridoids such as 3,4-DHPEA-EDA and verbascoside are the most abundant compounds in OVW (Servili *et al*., 2004). The pollution potential of OVW strictly dependent by the polyphenols' content and expressed as biochemical demand of oxygen (BOD_5) ranges from 35 to 110 g/L, while the chemical demand of oxygen (COD) ranges from 40 to 196 g/L (Niaonakis and Halvadakis, 2004); therefore the recover of the large amount of OVW phenols seems an interesting way to give an added value to a product that represents a disposing cost for the olive oil industries (Roig *et al*., 2006). At this regard several approaches have been already developed (Turano *et al.,* 2002; kujawski *et al*., 2004; Agalias *et al*., 2007; Paraskeva *et a*l., 2007; Roig *et al.*, 2006; Russo, 2007; Khoufi *et al*., 2008; Gortzi *et al*., 2008) although there are different constrains to utilize the proposed processes on a plant scale, because of their complexity that requires a OVW pre-treatment as well as their high costs of treatment and plant installation. As shown in Fig. 6 recently a membrane filtration system has been applied in an industrial scale plant to obtain a crude phenolic concentrate (CPC) from an OVW after a pre-treatment with a depolymerising enzymatic pool (Servili *et al*., 2011a). This process permits an OVW volume reduction ranged between 75 and 80% and a wide decrease of the original OVW pollution load (more than 95%). The obtained CPC has a concentration in polyphenols four times greater than that of the initial OVW content, among which the 3,4-DHPEA-EDA and the verbascoside are those present in higher concentrations, although the 3,4-DHPEA-EDA content is strongly conditioned by the OVW prolonged storage time because of its hydrolysis (Servili *et al.*, 2011a).

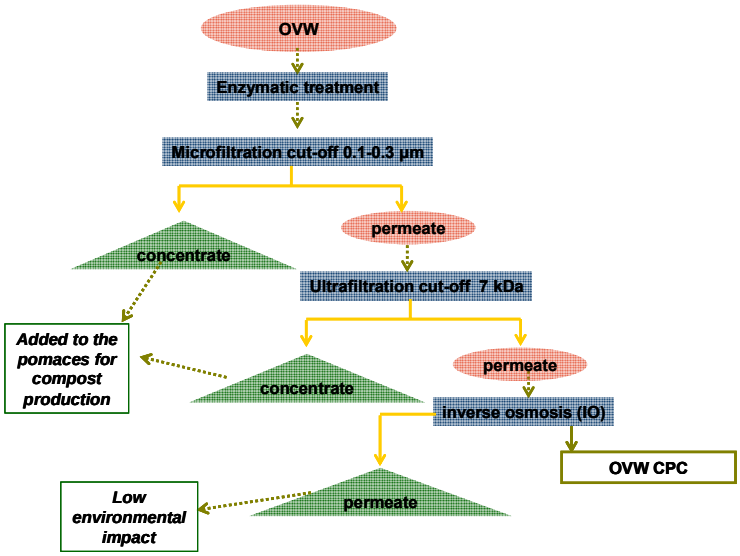

Fig. 6. Flow-chart of CPC production by OVW membrane treatment. Legend: OVW = olive-vegetation water, CPC = crude phenolic concentrate. (Servili *et al*., 2011a).

According to Servili *et al*. (2011a) the phenolic concentrate shows numerous potential uses that include the recycle in the oil mechanical extraction process to obtain EVOO enriched with hydrophilic phenols. Further application of the CPC can for the production of functional foods, enriched with bioactive phenols characterized by the same biological activities observed for the EVOO hydrophilic phenols (Servili *et al.*, 2011b).

To the secoiridoids a high antimicrobial activity is also recognized, especially against pathogenic species (Cicerale *et al*., 2011; Obied *et al*., 2012). These substances, represent an opportunity as alternative or complementary to the conventional additives for food with stabilizing, antioxidant and antimicrobial activities.

Acknowledgements

The authors wish to thank the Consorzio Olivicolo Italiano UNAPROL – Italy (Projects Reg. EC Nos. 2080/2005 and 867/2008) for the financial support.

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