Olive paste fast preheating and the quality of extra virgin olive oil: sensory and chemical markers

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ABSTRACT

The virgin olive oils (EVOOs) obtained with fast preheating of the olive paste after crushing combined or not with different malaxation periods were studied by means of multivariate statistical elaboration in order to find correlations between the chemical data and the sensory attributes and oxidative stability during a storage period of 12 months. Principal Component Analysis showed that a VOO with high content of simple phenolics compared to the complex phenolics is an oil which has been subjected to oxidation, for instance due to the storage or excessive heat. Based on these results, a new chemical index of 'normalized tyrosols' calculated as the ratio between the sum of concentration of 3,4-dihydroxyphenylethanol (3,4-DHPEA) and 4-hydroxyphenylethanol (p-HPEA) and the sum of total phenols (determined with HPLC) was proposed for the first time. This index is highly positively correlated to primary oxidation (r=0.6941) and inversely related to the oxidative stability (r=-0.6025). In addition, the normalized tyrosols are a good marker of sensory defects of EVOOs.

Szybkie ogrzewanie masy oliwkowej i jakość oliwy z oliwek z pierwszego tłoczenia: wskaźniki sensorowe i chemiczne

Słowa kluczowe: oliwa z oliwek z pierwszego tłoczenia, ogrzewanie, rozcieranie, związki fenolowe, HPLC, analiza sensorowa, preferencje konsumentów

STRESZCZENIE

Badano oliwę z oliwek z pierwszego tłoczenia (EVOOs) otrzymaną przy szybkim ogrzewaniu masy oliwkowej po rozcieraniu połączonym lub nie z różnym czasem mieszania, za pomocą wielowymiarowego opracowania statystycznego, w celu znalezienia korelacji między danymi chemicznymi a cechami smakowymi oraz odpornością na utlenianie podczas przechowywania przez 12 miesięcy. Analiza głównych składowych wykazała, że VOO z dużą zawartością prostych związków fenolowych, w porównaniu ze złożonymi związkami fenolowymi, jest oliwą podatną na utlenianie, np. podczas długotrwałego przechowywania lub przy nadmiernym ogrzewaniu. Na podstawie otrzymanych wyników zaproponowano znormalizowany indeks tyrozolowy, obliczony jako stosunek sumy stężenia 3,4-dihydroksyfenyloetanolu i 4-hydroksyfenyloetanolu oraz sumy wszystkich fenoli (oznaczonych za pomocą HPLC). Ten indeks jest wprost proporcjonalny do pierwotnego utleniania (r=0,6941) i odwrotnie proporcjonalny do odporności na utlenianie (r=-0,6025). Znormalizowany indeks tyrozolowy jest dobrym wskaźnikiem wad sensorowych EVOOs.

1. INTRODUCTION

The oil produced from the fruits of *Olea europaea* with physical means such as pressure or centrifugation and without any chemical treatment is defined as virgin olive oil (VOO). Over the past decade, the consumption of olive oil has decreased in three world main producing countries (Italy, Spain and Greece), whereas it is increasing in most of Central and Northern Europe, particularly in Poland [1]. In the EU, the sensory properties of VOO (intensity of the defects perceived, their fruitiness and other positive attributes) are used to grade the quality of a VOO on the basis of an official organoleptic assessment by a trained panel [2]. Moreover, the sensory quality is particularly important in VOO for marketing reasons, especially in countries where virgin olive oil has become more and more popular in relatively recent times.

The processing operations related to the extraction of virgin olive oil (VOO) are fundamental steps affecting the final nutritive and sensory quality of the product and its stability [3]. There are several factors, particularly related to olive harvest and technological operations of crushing and kneading that play a crucial role in determining the total quantity of phenolic compounds and their profile [4-6].

Malaxation or kneading (the slow mixing of the olive paste after milling) is a crucial step in the production of a high quality VOO [6]. The malaxation time and especially temperature significantly affect the overall quality of VOO [7], because they can influence the total amount and profile of selected microconstituents, such as the hydrophilic antioxidants present in the olive paste which diffuse into the VOO [8, 9]. These minor polar compounds are strictly related both to the sensory properties and to the oxidative stability of VOO upon thermal treatment and during the domestic storage [10].

During the crushing and malaxing steps, enzymatic oxidative reactions occur. The enzymes involved in the catalysis of most part of these reactions are lipase, lipoxygenase, polyphenol oxidase (PPO) and peroxidase (POD) [11]. When the drupe is damaged for whatever event (mechanical injury, bruising, heating, wrinkling, overripening and crushing), the enzymes get in contact with the oil drops being their substrate of action. Thus, oxidation, peroxidation and lipolysis can be triggered and can proceed further during kneading [12]. Lipoxygenase and peroxidase cause the formation of hydroperoxides, many of which will turn into volatile compounds (alcohols and aldehydes up to six carbon atoms and their corresponding esters), through a cascade of other enzymes. These volatile compounds are responsible for the characteristic aroma of olive oil [13-16]. However, part of the neo-formed hydroperoxides will react with the natural antioxidants present in

the oil. Consequently, the combined action of lipoxygenase, peroxidase and polyphenol oxidase can limit the quantity of antioxidants left in the final product.
As demonstrated by Angerosa and Di Giacinto [17] and Alloggio et al. [18], the crushing method has no influence on the quality parameters (free acidity, peroxide value, spectrophotometric constants, global sensory evaluation), but affects the final amount of polyphenols and consequently the oxidative stability and the perception of bitter and pungency sensation.
Also the kneading parameters can affect the volatile fraction and the phenolic profile of olive oil due to the reactions of the 'lipoxygenase pathway'. Although the phenolic compounds can be already present in the endocellular oil, the kneading causes both qualitative and quantitative modifications [19]. In particular, the endogenous enzymes POD and PPO can oxidize secoiridoids and lead to a decrease of the phenolic content of the oil; this turns out in the decrease of the bitter and pungent sensations and of the resistance towards oxidation of the oil. Together with the oxygen level and the time, the temperature is one of the parameters influencing the enzymatic activities. The temperature affects both the content of volatile compounds and also the extraction yield and the amount of phenols extracted.
Literature data reports conflicting results regarding the effects of the kneading temperature on the phenolic compounds [20]. There is, however, no influence of the temperature on the lignans [21]. This could be explained by the greater lipid character and less antioxidant activity of these molecules compared to other hydrophilic phenols [22]. A fast preheating step of the olive paste after olive crushing has been found to be an interesting strategy on an industrial scale [20, 23]. It allowed obtaining a VOO with a tailor-made bitter/pungent attribute from olive varieties which are naturally rich of phenolic compounds. Therefore, the resulting VOO can better meet the taste and preference of targeted groups of consumers, especially in Central and Northern Europe (e.g. Poland).
In the present study, the oils obtained with fast preheating of the olive paste after crushing combined or not with different malaxation periods (described in a previous research work [20]) were studied by means of multivariate statistical elaboration in order to correlate the chemical data with the sensory attributes and oxidative stability during a storage period of 12 months.

2. EXPERIMENTATION

2.1 Olive oil production and storage

As reported by Fiori et al. [20] the experimental tests were performed by processing a blend of olives of the cultivar Frantoio and Leccino in the same proportion (600 kg) with a "modified" two-phase continuous plant (Pieralisi Group, Jesi, Italy). The olives were harvested, defoliated and washed before being processed. The olives were processed by using a system consisting of a preheather, a mobile hammer crusher and a malaxer (Genius P4 model, Pieralisi Group, Jesi, Italy). Then, the oil was extracted by means of a horizontal centrifuge (decanter) operating at 2410 g (Maior 'special' model, Pieralisi Group, Jesi, Italy), and the separation of the resulting oily must was achieved in a vertical discharge centrifuge.
Three tests [20] were developed in order to monitor the effects of:
- the pre-heating of the olive paste after olive crushing with (sample Pr) and without (sample Pf) malaxation (Experiment 1);
- the reduction of the malaxation time to 10 min (Pr10) after pre-heating for 72 sec (Experiment 2);
- the different transit periods (102 sec for sample Pf35; 72 sec for sample Pf50; 48 sec for sample Pf75) of the olive paste inside the pre-heater without malaxation (Experiment 3).
A control sample was produced in every experiment (Mc1, Mc2 and Mc3). The pre-heather (Pieralisi Group S.p.A., Jesi, AN, Italy) was a cylindrical segment (6 m length with a 16 cm internal diameter) with an inner cavity for the passage of the olive paste by means of a screw feeder.
Two bottles for each analysis time (T0, T3, T6 and T12) were prepared during each experimental procedure. The bottles (750 mL) were sealed with a screw cap and kept in the dark and at room temperature for the entire period of experimentation (12 months). In the present work, the bottles sampled at time 0, 3 and 12 months were taken into consideration.

2.2 Determination of legal quality parameters

Free acidity (% oleic acid/100 g olive oil) and peroxide level (meq O2kg^{-1} oil) were carried out for each oil sample according to the European Commission [23, 24] and the International Olive Council [25] standard methods, as reported in Fiori et al. [20]. The sensory evaluation was carried out by eight judges who were fully trained in the evaluation of VOO according to the official methods of the EC Reg. n. 640/2008. All these determinations were performed in the oils after 0, 3 and 12 months, respectively.

2.3 Determination of the oxidative stability

The oxidative stability was determined with the Rancimat apparatus (Metrohm model 679, Herisau, Switzerland). The oil samples (5 g each) were heated to 110°C under an air stream at 20 L h^{-1}. The oxidation products were conveyed by the air flow in distilled water (60 mL) and measured through the change of conductivity of water, monitored by an electrode. The induction period was determined by drawing the two tangents of the time-conductivity curve and projecting the intersection onto the time-axis. The induction period was expressed in h.

2.4 Extraction of the phenolic fraction

The phenolic compounds were extracted according to the procedure described in earlier research works [26, 27]. The extracts were resuspended in 1 mL methanol and the solutions were filtered through 0.2 µm regenerated cellulose filters (Schleicher & Schuell, Dassel, Germany) and stored at -20°C before analysis.

2.5 Spectrophotometric determination of total phenols

The total phenols content of the hydroalcoholic extract was determined according to the spectrophotometric method reported by Singleton and Rossi [28] by using a CARY 5000 UV-Vis-NIR (Varian, Leinì, Italia) at 765 nm. The results were expressed as gallic acid equivalents (mg kg^{-1} oil) based on the calibration curve (r^2=0.999). Folin-Ciocalteu reagent and gallic acid were obtained from Merck & Co. Inc. (Darmstadt, Germany).

2.6 HPLC-DAD/ESI-MS2 determination of phenolic compounds

The identification of the phenolic profile was performed by high performance liquid chromatography coupled to a photodiode detector (DAD) (Varian Prostar 330 PDA) and a mass spectrometer (Thermoquest, San José, CA, USA). An aliquot of 20 µL was injected into a HPLC system consisting of a ternary pump (Varian 9010, Walnut Creek, CA, USA), and a column (25 cm length x 4.6 mm internal diameter) packed with C18 stationary phase (5 µm particle size) (Chromospher, Middelburg, The Netherlands). The total run time was 75 min, with a mobile phase flowing at 0.8 mL/min. The gradient elution consisted of water/acetic acid (98:2 v/v) as mobile phase A and methanol as mobile phase B [27].

The chromatograms were registered at three different wavelengths: 280, 320, and 345 nm. Each wavelength was suitable for a peculiar class of compounds: 280 nm was used for phenyl ethyl alcohol and secoiridoids, 320 nm for hydroxycinnamic acids and 345 nm for flavones. The data were acquired using the Varian Star 6.3 software. For structural elucidation, the HPLC system was coupled on-line with an LCQ ion trap mass spectrometer with an ESI (Electrospray Ionization) interface. The effluent (0.1 mL/min) entered the mass spectrometer through a capillary set to 4.4 KV and 200°C. Second order mass experiments (MS2) were conducted using a collision energy of 30-40%. The spectra were acquired in negative ionization with a mass range 50-1000 amu, by using the Excalibur software version 1.2 (Thermoquest, Milan, Italy).

Simple phenolic compounds, secoiridoids and flavones were quantified according to calibration curves obtained with p-dihydroxyphenyl ethanol, oleuropein and apigenin (all with r^2=0.999), respectively.

2.7 Statistical analysis

The correlation matrix was obtained by using GraphPad InStat ver. 3.05 (GraphPad Software, San Diego, CA, USA). Principal Component Analysis was carried out by using The Unscrambler v 7.6 (Camo Inc., Corvallis, OR).

3. RESULTS AND DISCUSSION

The fast preheating of the olive paste after crushing was performed in order to promote the fluidization of the oil and consequently the coalescence of the oil droplets in a very quick time (ranging 48-102 sec) by using a new geometry of equipment which allows a faster heat transfer between the metal surface of the heating cylinder and the product, compared to a conventional malaxer, which consists of a large mixing chamber where the olive paste is usually slowly churned for a much longer time (30-45 min). The reduction of the malaxation time combined with this fast olive paste preheating stage decreased the contact time between the degradative enzymes and their substrates (polyphenols or lipids). At the same time, the oil yield was not significantly modified, as described by Fiori et al. [20]. For these reasons, the oils obtained with the experiments described in this work showed different qualitative characteristics.

3.1 HPLC-DAD/ESI-MS2 of phenolic compounds

The phenolic fingerprint of a VOO sample is shown in Figure 1. The chromatographic trace was obtained with HPLC coupled to a diode array detector (DAD) and the identification was confirmed by means of structural information from mass spectrometric experiments of first and second order according to Fiori [29] and Boselli et al. [26]. The identified molecules were low molecular weight phenolics such as hydroxytyrosol, tyrosol, vanillic acid and hydroxytyrosol acetate, and more complex compounds such as the secoiridoids which are typical of virgin olive oil (among them, decarboxymethyl oleuropein aglycone, oleuropein aglycone, decarboxymethyl ligstroside aglycone, ligstroside aglycone, oleuropein aglycone, ligstroside aglycone) and also flavones (apigenin and luteolin). Lignans were under the detection limit of the method.

3.2 Principal Component Analysis (PCA) of the samples across the chemical and sensory variables

Principal Component Analysis was used in order to find potential positive and negative correlations among the sensory properties of the oils (sensory panel) and the chemical parameters (phenolic compounds detected with HPLC, oxidative stability and chemical legal parameters). This

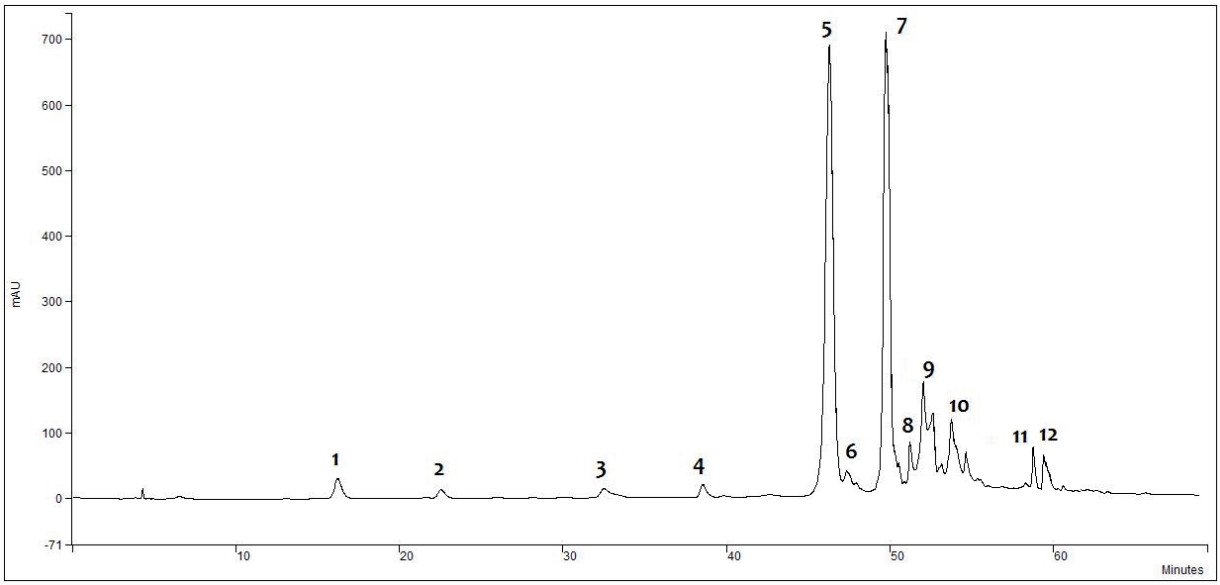

Figure 1 HPLC-DAD (λ=280 nm) chromatographic trace of a VOO sample. 1: hydroxytyrosol (3,4-DHPEA); 2: tyrosol (p-HPEA); 3: vanillic acid; 4: hydroxytyrosol acetate (3,4-DHPEA Ac); 5: decarboxymethyl oleuropein aglycone dialdehydic form (3,4-DHPEA-EDA); 6: oleuropein aglycone dialdehydic form (DOA); 7: decarboxymethyl ligstroside aglycone dialdehydic form (p-HPEA-EDA, oleocantal); 8: ligstroside aglycone dialdehydic form (DLA); 9: oleuropein aglycone (3,4-DHPEA-EA); 10: ligstroside aglycone (LA); 11: luteolin; 12: apigenin

Rysunek 1 Chromatogram HPLC-DAD (λ=280 nm) próbki VOO. 1: hydroksytyrozol (3,4-DHPEA); 2: tyrozol (p-HPEA); 3: kwas wanilinowy; 4: octan hydroksytyrozolu (3,4-DHPEA Ac); 5: dekarboksymetylooleuropeina w postaci aglikonu dialdehydowego (3,4-DHPEA-EDA); 6: oleuropeina w postaci aglikonu dialdehydowego (DOA); 7: ligstrozyd dekarboksymetylowy w postaci aglikonu dialdehydowego (p-HPEA-EDA, oleoktantal); 8: ligstrozyd w postaci aglikonu dialdehydowego (DLA); 9: aglikon oleuropeiny (3,4-DHPEA-EA); 10: aglikon ligstrozydu (LA); 11: luteolina; 12: apigenina

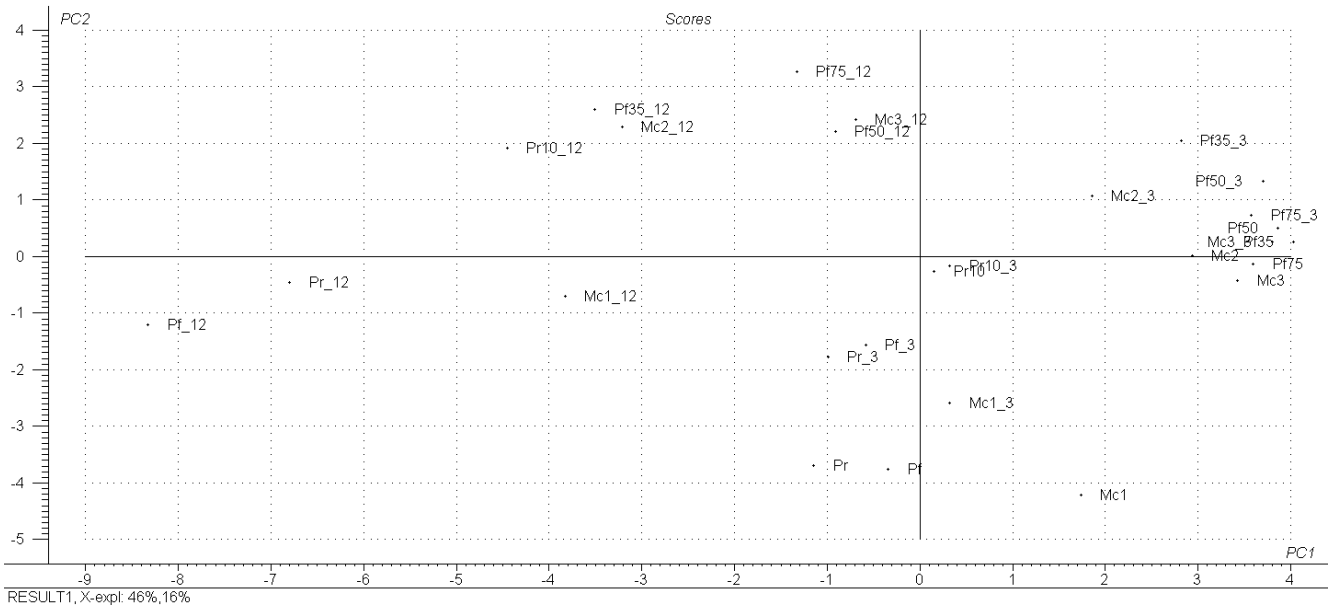

Figure 2A Score plot of the VOO samples. VOO of Experiment 1 are Mc1 (control), Pr and Pf. Oils of Experiment 2 are Mc2 (control) and Pr10. VOOs of experiment 3 are Mc3 (control), Pf35, Pf50 and Pf75. Samples without underscore are fresh VOOs; the months of storage are reported as _3 and _12

Rysunek 2A Wykres wyników dla próbek VOO. VOO z doświadczenia 1 to Mc 1 (kontrolna), Pr i Pf. Oliwy z doświadczenia 2 to Mc 2 (kontrolna) i Pr 10. VOOs z doświadczenia 3 to Mc 3 (kontrolna), Pf 35, Pf 50 i PF 75. Próbki bez podkreśleń to świeże VOOs. 3 i 12 oznaczają miesiące przechowywania

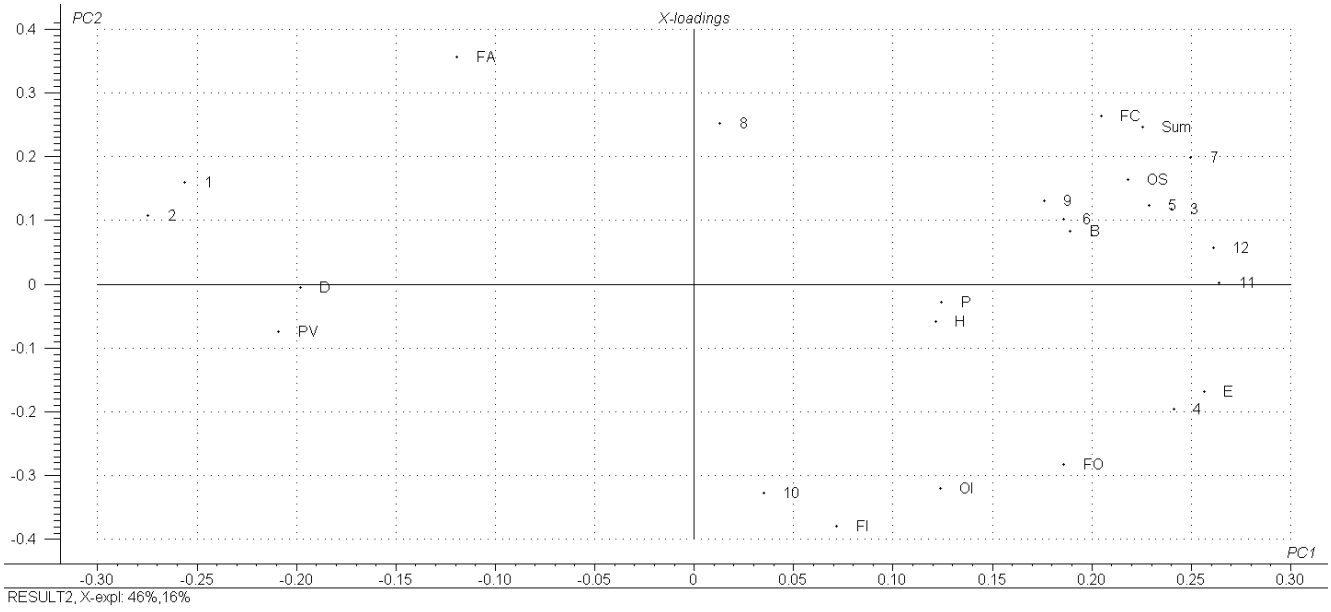

Figure 2B Loading plot of the variables of the VOOs. Sensory attributes: Ol, olfactory intensity; FO, fruity (of olive) intensity; H, intensity of herbaceous; Fl, fruity intensity; D, defects; B, bitter; P, pungency; E, equilibrium. Chemical determinations: 1-12 are the phenolic compounds listed in Fig. 1; PV, peroxide number; FA, free acidity; FC, Folin index; Sum, sum of HPLC phenolic compounds; OS, oxidative stability

Rysunek 2B Wykres obciążenia zmiennych VOOs. Cechy sensoryczne: Ol: intensywność zapachu; FO: intensywność owocowa (oliwy); H: intensywność ziołowa; Fl: intensywność owocowa; D: wady; B: gorzkość; P: ostrość smaku i zapachu; E: równowaga. Oznaczenia chemiczne: 1 – 12 to związki fenolowe przedstawione na Rysunku 1; PV – liczba nadtlenku; FA – kwasowość wolna; FC – indeks Folina; suma – suma związków fenolowych oznaczonych za pomocą HPLC; OS – stabilność oksydacyjna

is still a challenging subject for the evaluation of the overall quality of VOO and also for the physiological aspects related to food perception and acceptance. In fact the relationships between chemical composition and sensory organs must be still completely understood. The resulting score plot of the samples and the loading plot of the variables are reported in Figure 2A and 2B, respectively.

From Figure 2B it is clear that the oxidative stability (OS) of the VOOs was directly related to the sum of phenolic compounds either determined via HPLC (Sum) or via the Folin method (FC). Figure 2A shows the dependence of the sum of phenolics and oxidative stability from the storage time; in fact the samples at 12 months are concentrated on the left part of the plot, whereas the 0 months and 3 months tend to cluster in the center and right side of the score plot. The direct correlation between phenolics and resistance towards oxidation has been already described in previous research works conducted on different EVOO by the same group [27].

However, another very interesting result must be reported. The phenolic compounds 1 (tyrosol) and 2 (hydroxytyrosol) plotted in Figure 2B were related to a sensory defect (D) and to high peroxide value (PV) and to high free acidity (FA) as also reported in Figure 3 showing the correlation matrix. Conversely, the other phenolic compounds were related to all the positive sensory attributes of VOO (Fig. 2B).

It should not be surprising that a high content of tyrosol and hydroxytyrosol (natural antioxidants) can be related to oxidation and sensory defect. This is a confirmation of evidence that was already reported in previous research [27]. As an additional information, Figure 4 shows the positive correlation between the normalized sum of tyrosol and hydroxytyrosol (vs the sum of HPLC phenolics) and the extent of primary oxidation determined with the peroxide number; the correlation coefficient r was 0.6941 (corresponding to r^2=0.482), as reported in Figure 3. This value is quite high considering the extreme complexity of VOO matrix (it is not a standard solution but a real world sample). This correlation also showed that a VOO with high content of simple phenolics compared to the complex phenolics is an oil which has been subjected an oxidation, for instance due to the storage or excessive heat. In other words, the lysis of complex phenolics in a medium with high free acidity (FA) lead to the release of tyrosol and hydroxytyrosol during the 1-year storage especially in the EVOOs which were subjected to excessive preheating treatment.

Correlation Matrix Results

	A:	B:	C:	D:	E:
A:1+2	1.0000	-0.5050	0.9524	0.5209	0.5462
B:sum	-0.5050	1.0000	-0.6074	-0.5873	-0.0244
C:(1+2)/sum	0.9524	-0.6074	1.0000	0.6941	0.4497
D:PV	0.5209	-0.5873	0.6941	1.0000	0.2007
E:FA	0.5462	-0.0244	0.4497	0.2007	1.0000
F:OS	-0.5072	0.8553	-0.6025	-0.6001	-0.2316

	F:
A:1+2	-0.5072
B:sum	0.8553
C:(1+2)/sum	-0.6025
D:PV	-0.6001
E:FA	-0.2316
F:OS	1.0000

Each correlation coefficient (r) is calculated independently, without considering the other variables.

Figure 3 Correlation matrix of selected variables. 1+2, sum of concentration of tyrosol and hydroxytyrosol; sum, sum of HPLC phenolics; (1+2)/sum, normalized tyrosols; PV, peroxide number; FA, free acidity; OS, oxidative stability

Rysunek 3 Macierz korelacyjna wybranych zmiennych. 1 + 2, suma stężeń tyrozolu i hydroksytyrozolu; suma, suma związków fenolowych oznaczonych za pomocą HPLC; (1 + 2)/suma, znormalizowane tyrozole; PV, liczba nadtlenku; FA, kwasowość wolna; OS, stabilność oksydacyjna

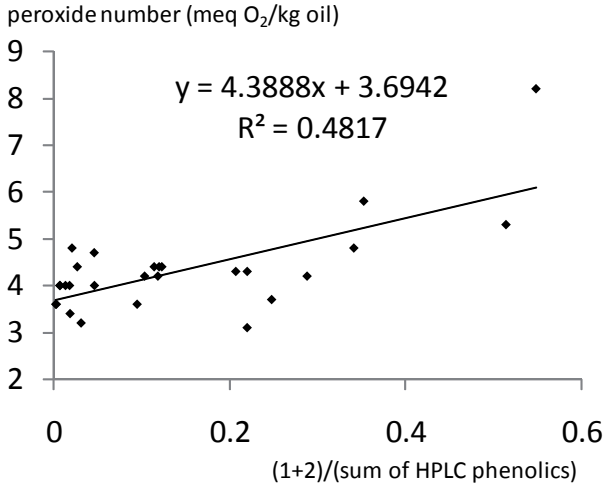

Figure 4 Correlation between the normalized sum of tyrosol (1) and hydroxytyrosol (2) and the extent of primary oxidation

Rysunek 4 Zależność między znormalizowaną sumą tyrozolu (1) i hydroksytyrozolu (2) a zakresem utlenienia pierwotnego

4. CONCLUSIONS

In the context of the extraction process of VOO, the temperature and malaxation conditions represent a fundamental parameter in determining the quality of the final product, evaluated both in the fresh oil and during the first 12 months of storage.

The use of the preheater can determine an accelerated *maturation or ageing* of the final VOO which can be deleterious if the temperature is not controlled during the processing operations. This evidence suggests that the use of the preheater with low temperatures may lead to a quality advantage, particularly related to the phenolic content. In fact the fast preheater could be very useful on an industrial scale in order to obtain a high qualitative VOO with a predefined phenolic content according to the preference of targeted consumers who do not appreciate too bitter and too pungent oils (Northern and Central Europeans, for instance).

'Bitter' (B) and 'pungent' (P) are positive attributes in the specific vocabulary of the organoleptic assessment of VOO and are always positively correlated with the oxidative stability and to the phenolic content. However, a high content of tyrosol and hydroxytyrosol compared to complex phenols is related to primary oxidation. For this reason, the new chemical index of 'normalized tyrosols' as the ratio between the sum of concentration of 3,4-DHPEA and p-HPEA and total phenols was presented for the first time. This index is highly related to peroxide number, free acidity and inversely related to the oxidative stability. In addition, the normalized tyrosols are a good marker of sensory defect of EVOOs.

5. ACKNOWLEDGEMENTS

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