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Effects of olive paste fast preheating on the quality of extra virgin olive oil during storage

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ABSTRACT

The olive paste obtained after crushing was fast preheated under different time/temperature conditions and then malaxed in an industrial oil mill (600 kg Frantoio/Leccino olive blend). Legal parameters (peroxides, free acidity and sensory panel), oil yield, total phenolic content, oxidative stability and phenolic profile were monitored during 12 months of storage of the virgin olive oil (VOO) kept in closed bottles in the dark. A fast preheating not longer than 72 s at 38 °C without malaxation lead to an extra VOO with a shelf-life of at least 12-months, similarly to the traditional EVOO obtained with malaxation. A fast preheating not longer than 72 s at 38 °C followed by 10 min malaxation lead to an EVOO with a 'mild' sensory profile and a shelf life of at least 12-months. Thus, the use of a specific designed fast preheater instead or before (a shortened) malaxation allows to obtain an EVOO with a low bitter/pungent attribute from olives which are rich of (sometimes unpleasant) phenolic compounds with the aim to meet the preference of targeted groups of consumers. Time and temperature of fast preheating are the critical parameters of the process.

1. Introduction

Many investigations deal with the influence of technological operations of olive processing (crushing and malaxation/kneading) which play a crucial role in determining the oil yields and quality (Amirante, Clodoveo, Tamborrino, Leone, & Paice, 2010; Clodoveo, 2013a, 2013b; Morales, Angerosa, & Aparicio, 1999; Servili & Montedoro, 2002). During malaxation, the endogenous enzymes POD and polyphenoloxidase (PPO) can oxidize secoiridoids and reduce the concentration of oil phenolics, thus decreasing the bitter and pungency attributes and the oxidative stability of the resulting oil (Angerosa, Mostallino, Basti, & Vito, 2001; Georgalaki et al., 1998). Typically, higher temperature increases the oil yield because it reduces the oil viscosity and promotes the aggregation of the oil droplets (Inarejos-García, Gómez-Rico, Salvador, & Fregapane, 2009). However, Ranalli and coll. (Ranalli, Contento, Schiavone, & Simone, 2001) suggested a kneading temperature not higher than 30 °C as they found a general deterioration of the oil quality at 35 °C, without any substantial increase of the extraction yield. Furthermore, Kalua and coworkers (Kalua, Bedgood, Bishop, & Prenzler, 2006) showed that a temperature increase up to 45 °C resulted in a significant lowering of the yield, compared to temperatures of 15 and 30 °C. The temperature range 30–36 °C was reported to be even negatively correlated with the content of phenolic compounds, as suggested by Parenti, Spugnoli, and Cardini, (2000). They showed that the combination of temperature/phenolic compounds presented a bell-shaped trend with a maximum at 27 °C. These results could be justified by the fact that the enzymes PPO and POD have an optimal temperature between 30 and 40 °C (Ünal, Taş, & Şener, 2011) and around 30–35 °C (Saraiva, Nunes, & Coimbra, 2007), respectively. Older literature data have reported an inverse relationship between the temperature and the phenolic content (Angerosa et al., 2001; Servili, Selvaggini, Taticchi, Esposto, & Montedoro, 2003). More recent research showed an increase of the phenolic fraction in response to a temperature increase (Boselli, Di Lecce, Strabbioli, Pieralisi, & Frega, 2009; Kalua et al., 2006). Recently, Esposto et al. (2013) tested the introduction of a heat exchanger before malaxation because the traditional malaxation process has low thermal transfer efficiency, and for this reason, the thermal conditioning of

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olive pastes is relatively long compared to the optimal processing temperature. This aspect influences the activity involved in oil extraction of the endogenous enzymes cited above. They found that optimal operative conditions (time and temperature) applied during malaxation after the flash thermal conditioning treatment of the olive paste can be opportunely chosen for improving the relative virgin olive oil quality. Another option to realize a more efficient heat exchange between the small malaxer surface area and the large volume of olive paste is the application of ultrasound, as proposed by Clodoveo, Durante, La Notte, Punzi & Gambacorta (2013). Clodoveo, Durante, & La Notte (2013) combined an ultrasound probe with a double-pipe heat exchanger and patented a method and apparatus for thermal conditioning of olives or other oleaginous fruits combined with a crushing and kneading system in controlled or modified atmosphere (Clodoveo, 2013c).

In the present study, we have investigated the effects of a fast preheating treatment of the olive paste (combined or not with malaxation) on the quality parameters, oxidative stability and phenolic profile of VOO. The quality of VOOs was monitored during a storage period of 12 months under standard conditions.

2. Materials and methods

2.1. Olive oil extraction and storage

A blend of defoliated and washed olives of the *cultivar* Frantoio and Leccino in the same proportion (600 kg) were processed with a "modified" two-phase continuous plant (Pieralisi Group, Jesi, Italy). The system consisted of a fast pre-heater, a mobile hammer crusher and a malaxer (Genius P4 model, Pieralisi group Jesi, Italy). Successively, the oil was extracted using a horizontal centrifuge (decanter) operating at 2410 g (Maior "special" model, Pieralisi Group, Jesi, Italy). Three different experiments (Fig. 1) were carried out in order to evaluate the effects of fast pre-heating of the olive paste after olive crushing (Experiment 1); the reduction of the malaxation time after preheating (Experiment 2); and the different transit periods of the olive paste inside the preheater (Experiment 3). The three experiments were performed on different days of the oil campaign, respectively on November 3, after 22 days and after 28 days. A control sample was produced in each experiment; they were obtained from a not-preheated olive paste that was malaxed for 35 min (MC_1, MC_2 and MC_3). The preheater was a cylindrical segment with an inner cavity for the passage of the olive paste by means of a screw feeder. It was 6 m long and was sorrounded by a hollow space (16 cm internal diameter) in which hot water ($T = 62$ °C) flowed in counter current to the passage of the olive paste. With Experiment 1 (Fig. 1), two experimental samples (Pr and Pf) were produced by transmitting a frequency of 50 rpm to the preheater. Successively, the olive paste was either malaxed for 35 min and then sent to decanter (sample Pr). In the other case (sample Pf), the olive paste was sent directly to the decanter, just after filling the standstill malaxer. The olive paste sample of Experiment 2 (Pr_10′) was malaxed for 10 min after preheathing and then sent to the decanter. The three samples of Experiment 3 (Pf_35, Pf_50 and Pf_75) were obtained with different transit time by varying the frequency transmitted to the preheater (as reported in Fig. 1). Before entering the decanter, the temperature of all the olive paste samples was measured. Two bottles for each analysis time (T0, T3, T6 and T12) were filled with the oil samples prepared during each experimental procedure. The bottles (750 mL) were sealed with a screw cap and were kept in the dark and at room temperature for the entire period of experimentation (12 months). Olive pomaces were also sampled within each production process.

2.2. Oil content of the pomace

The residual olive oil was extracted from different samples of pomace. Before extraction, the pomace samples (100 g) were freeze-dried (Virtis Freeze Dryer, Gardner, USA) in order to optimize the oil recovery. Then, 40 g of the freeze-dried sample were mixed with anhydrous sodium sulphate, placed in an extraction thimble and subjected to extraction of oil with n-hexane by using a Soxhlet apparatus (B-811, Büchi Labortechnik AG, Switzerland) for 8 h. The residual hexane was removed by means of a rotary evaporator at 30 °C (R-114, Büchi Labortechnik AG, Switzerland).

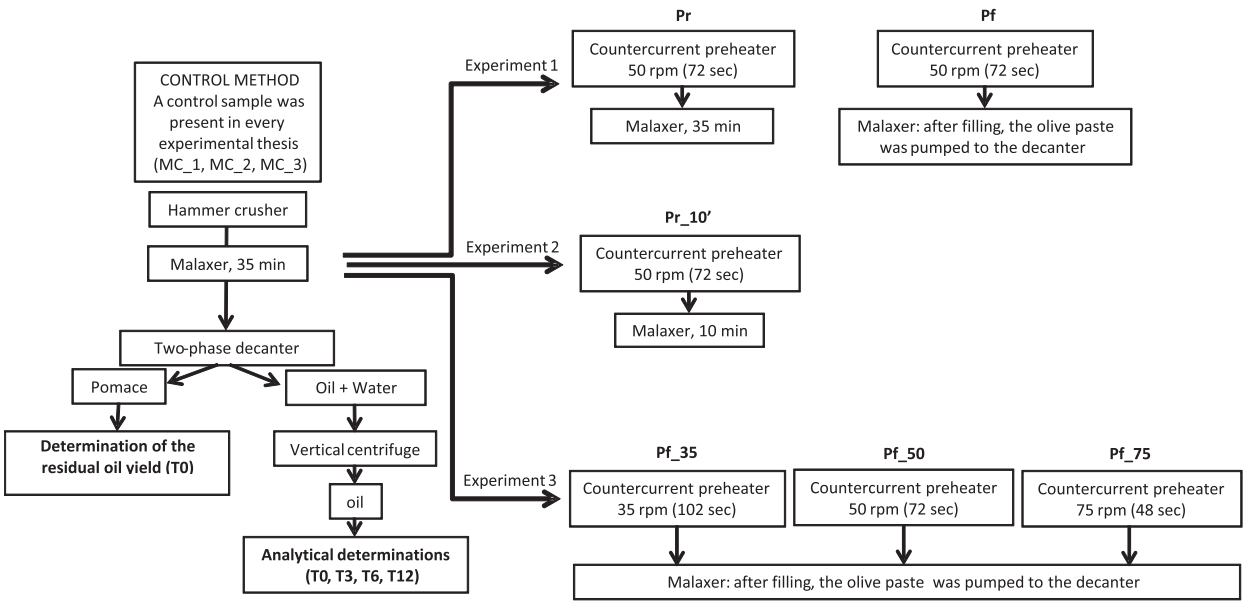

Fig. 1. ExtraVirgin Olive Oil processing protocols and sampling plan. In the three experiments, the 35-min malaxing process was replaced by the process described on the right part of the figure. Experiment 1: preheating of the olive paste; Experiment 2: reduction of the malaxation time of the preheated olive paste; Experiment 3: evaluation of different transit times of the olive paste inside the preheater.

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2.3. Determination of legal quality parameters and oxidative stability

Determination of free acidity (g oleic acid/100 g olive oil), peroxide level (meq O_2 kg^{-1} oil) and panel test were performed according to the official European Commission methods (EC, 1991; EC, 2003; EC, 2008; respectively). The determinations were performed at each analysis time (T0, T3, T6 and T12) except the sensory evaluation that was carried out at T0, T3 and T12. A sensory profile for each sample was obtained by each of the eight judges; the medians were calculated and reported as radar charts. The oxidative stability was determined with a 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 induction period was determined by drawing the two tangents of the time–conductivity curve and projecting the intersection onto the time-axis.

2.4. Analysis of the phenolic fraction

The phenolic compounds were extracted and determined according to the procedure described by Boselli et al. (2009). Briefly, the methanolic extract of minor polar compounds was used for the spectrophotometric determination of total phenols with the Folin–Ciocalteu reagent by using a CARY 5000 UV–Vis–NIR spectrophotomer (Varian, Leinì, Italia) at 765 nm. The results were expressed as gallic acid equivalents (mg kg^{-1} oil) based on a calibration curve ($r^2 = 0.999$). The HPLC determination of phenolic compounds was performed on the methanolic extract with a photodiode detector. Simple phenolic compounds, secoiridoids and flavones were quantified according to calibration curves obtained with *p*-dihydroxyphenyl ethanol, oleuropein and apigenin (all with $r^2 = 0.999$) standard substances, respectively. For structural elucidation, the HPLC system was coupled on-line with an LCQ ion trap mass spectrometer with an ESI (Electrospray Ionization) interface, as reported in the cited work by the same authors.

2.5. Statistical analysis

The chemical data were statistically processed with one-way ANOVA followed by the Tukey–Kramer test ($p < 0.05$).

3. Results and discussion

In this study, the reduction of the malaxation time combined with olive paste fast preheating was aimed to reduce the oxidative phenomena involving phenolic compounds; the impact of the two combined processes was evaluated on the quality of the final product. The rationale for the fast preheating treatment of the olive paste is the fluidization of the oil and consequently the coalescence of the oil droplets to increase the oil yield. The temperature of the processes (measured at T0) was monitored during the passage of the olive paste into the preheater and in some cases after the malaxation step, before the olive paste entered the decanter. Before flowing into the decanter, the temperature of the three control samples (MC_1, MC_2 and MC_3) ranged between 31 and 32 °C, whereas the experimental samples were in the range 37–43 °C. The use of the fast preheater thus determined a temperature increase ranging between 6 and 11 °C. The final temperature of the preheated olive paste samples was mostly influenced by the seasonal period of processing rather than the duration of the preheating process. When the harvest and processing of olives were postponed, a decrease of the temperature of the paste exiting the preheater was observed, presumably associated with a colder external temperature. The monitored temperature of Pr and Pf (Experiment 1), which were produced in the first days of November, was between 42 and 43 °C, being 4–6 °C higher than the temperature of the other two experiments which were conducted 3–4 weeks later (38 °C and 37–38 °C for Experiment 2 and 3, respectively). After 35 min malaxation, the preheated olive paste of the sample Pr cooled down of only 1 °C (from 42 °C to 41 °C). The shortest kneading time (10 min) of the sample Pr_10′ did not result in a wide variation in temperature compared to the value recorded after the preheating phase.

The residual oil content in the pomace of three different olive batches was in the range between 32 and 44 g oil kg^{-1} pomace, in agreement with previous literature data collected by using a continuous two-phase plant (Aguilera, Beltrán, Sanchez-Villasclaras, Uceda, & Jimenez, 2010; Di Giovacchino, Sestili, & Di Vincenzo, 2002). The use of the fast preheater in the extraction line exerted different effects in terms of yield, depending on the frequency of transmission and whether the kneading phase was carried out. The samples of pomace Pr and Pr_10′, obtained after preheating (50 rpm) and kneading for 35 and 10 min, respectively, were characterized (44.4 and 36.4 g oil kg^{-1} pomace, respectively) by a slightly lower oil content (−1.35% and −1.65%) compared to the respective control samples, thus indicating a relatively higher efficiency of the extraction process. More appreciable results were observed when comparing the extraction yields in Experiment 3. The transit time of the olive paste inside the preheater was directly related to the oil yield; in fact, the oil content of pomace decreased from 34.0 to 32.8 g oil kg^{-1} pomace and then to 32.2 g oil kg^{-1} pomace when the preheating time was increased from 48 s (Pf_75) to 72 s (Pf_50) and then to 102 s (Pf_35). An increase in the oil yield as a result of preheating was also observed by Cruz, Yousfi, Oliva, and García (2007). In their study, the olives were preheated prior to crushing by immersion in water at temperatures between 50 and 60 °C. No difference was observed between the samples Pf_50 and MC_3. Therefore, preheating the olive paste with a frequency of 50 rpm lead to the same oil yield obtained with a kneading time of 35 min. Finally, a lower extraction yield compared to the control was observed by using a fast preheating step not associated with malaxation (Pf_75). In this case, the oil content in the pomace (34.0 g oil kg^{-1} pomace) was 3.65% higher than the control (MC_3, 32.8 g oil kg^{-1} pomace).

3.1. Effects of the storage on the quality of Extra Virgin Olive Oil

The free acidity (Tables 1–3) was well below the legal limit (less than 8 g free oleic acid kg^{-1} oil) set up for the category of EVOO (European Commission 1991, 2003) during the storage period. Free acidity increased from T0 to T12, but not substantially; the T12 acidity values reached around 3 g free oleic acid kg^{-1} oil. The low level of acidity found after one year of storage was presumably associated with the good conditions of the olives and to appropriate storage conditions of the oil over time. The samples of Experiment 1 and 2 were in a range of free acidity that did not differ significantly with respect to their controls in most cases (Tables 1 and 2). The free acidity of the sample Pf_35 (Experiment 3, Table 3) was similar to the control sample during the 12 months. Also the acidity of Pf_50 was very similar to the control sample. In the first three months of storage, the sample Pf_75 had significantly lower acidity than the control. After 12 months, free acidity was statistically higher than the control. Also the peroxide number was much lower than the legal limit (20 meq O_2/kg oil) for EVOO (European Commission, 1991) in all the samples. The general trend during storage was an increase up to T6, the time at which the maximum value was reached. At T12, in all samples, except Pf (Experiment 1), peroxides decreased to values close to T0 showing a degradation of the primary oxidation products. At T3 and T6, the samples of

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Table 1
Chemical profile (mean, $n = 2$) of the virgin olive oils of Experiment 1 at different storage time (from T0 to T12 months). Phenolic compounds are reported in mg kg^{-1} of oil. Hydroxytyrosol (3,4-DHPEA); tyrosol (*p*-HPEA); hydroxytyrosol acetate (3,4-DHPEA Ac); decarboxymethyl oleuropein aglycone dialdehydic form (3,4-DHPEA-EDA); oleuropein aglycone dialdehydic form (DOA); decarboxymethyl ligstroside aglycone dialdehydic form (*p*-HPEA-EDA, oleocantal); ligstroside aglycone dialdehydic form (DLA); oleuropein aglycone (3,4-DHPEA-EA); ligstroside aglycone (LA). Sum of HPLC phenolics is the sum of phenolic concentration listed above. Free acidity as g free oleic acid kg^{-1} oil; peroxide index as meq O_2 kg^{-1} oil; oxidative stability in hours (induction time). Different letters in each column for each storage time represent significant differences ($p < 0,05$). The pooled standard deviation for each parameter is reported in the last line. MC_1, control (no preheating, 35 min malaxation); Pr, 72 s preheating and 35 min malaxation; Pf, 72 s preheating, no malaxation.

Storage time	Sample Exper.1	3,4-DHPEA	*p*-HPEA	Vanillic acid	3,4-DHPEA Ac	3,4-DHPEA-EDA	DOA	oleocantal	DLA	3,4-DHPEA-EA	LA	luteolin	apigenin	Sum of HPLC phenolics	Free acidity	Peroxide value	Total phenols	Oxidative stability
T0	MC_1	8.4^{ab}	6.20^{a}	0.72^{a}	5.6^{a}	57.6^{a}	0.85^{a}	61.1^{a}	0.91^{a}	5.8^{b}	5.69^{a}	1.72^{a}	0.85^{a}	155^{a}	1.6^{a}	3.6^{b}	227^{a}	37.6^{a}
	Pr	8.0^{b}	5.18^{a}	0.76^{a}	3.8^{a}	40.7^{b}	0.74^{a}	57.2^{a}	0.94^{a}	2.7^{c}	5.33^{a}	1.69^{b}	0.81^{a}	128^{b}	1.9^{a}	4.2^{a}	187^{b}	29.1^{b}
	Pf	9.7^{a}	5.98^{a}	0.71^{a}	5.3^{a}	35.5^{b}	0.70^{a}	58.7^{a}	0.98^{a}	8.4^{a}	5.16^{a}	1.51^{b}	0.84^{a}	133^{c}	1.8^{a}	4.2^{a}	185^{b}	25.8^{c}
T3	MC_1	9.6^{a}	9.16^{a}	0.79^{a}	3.9^{a}	56.8^{a}	2.53^{a}	62.7^{a}	0.98^{b}	9.06^{a}	6.95^{a}	1.93^{a}	0.87^{a}	165^{a}	1.8^{b}	4.4^{a}	231^{a}	28.0^{a}
	Pr	8.6^{a}	9.14^{a}	0.78^{a}	3.93^{a}	42.7^{b}	1.71^{b}	57.6^{a}	2.56^{a}	8.26^{a}	7.43^{a}	1.71^{a}	0.83^{a}	145^{b}	2.6^{a}	4.4^{a}	195^{b}	25.0^{b}
	Pf	9.3^{a}	8.36^{a}	0.77^{a}	4.54^{a}	42.3^{b}	2.78^{a}	57.7^{a}	2.11^{a}	10.1^{a}	7.28^{a}	1.68^{a}	0.85^{a}	148^{b}	2.1^{ab}	4.4^{a}	197^{b}	25.9^{b}
T6	MC_1	17.3^{a}	14.6^{b}	0.69^{a}	2.84^{a}	61.4^{a}	1.15^{a}	59.9^{a}	1.17^{b}	4.25^{a}	3.84^{a}	2.01^{a}	0.53^{a}	170^{a}	2.8^{a}	7.3^{a}	257^{a}	30.0^{a}
	Pr	19.0^{a}	17.7^{a}	0.67^{a}	2.88^{a}	38.1^{b}	1.03^{a}	53.7^{b}	1.38^{b}	4.11^{a}	2.50^{b}	1.97^{a}	0.52^{a}	144^{b}	2.8^{a}	7.0^{a}	203^{b}	24.2^{b}
	Pf	20.2^{a}	15.2^{ab}	0.65^{a}	3.24^{a}	32.1^{c}	1.19^{a}	46.9^{c}	2.01^{a}	3.90^{a}	3.81^{a}	1.98^{a}	0.53^{a}	132^{c}	2.5^{a}	6.9^{a}	180^{c}	26.2^{b}
T12	MC_1	23.2^{b}	22.89^{a}	0.41^{a}	1.67^{a}	32.9^{a}	1.12^{a}	45.1^{a}	1.08^{a}	2.79^{a}	2.52^{a}	1.44^{a}	0.37^{a}	135^{a}	2.9^{a}	4.8^{b}	214^{a}	31.2^{a}
	Pr	32.1^{a}	30.5^{b}	0.43^{a}	1.34^{a}	24.8^{b}	1.10^{a}	25.7^{b}	0.88^{a}	1.78^{b}	2.06^{b}	1.37^{a}	0.36^{a}	122^{b}	2.8^{a}	5.3^{a}	197^{a}	25.9^{b}
	Pf	31.4^{a}	31.6^{b}	0.39^{a}	1.66^{a}	18.3^{c}	1.15^{a}	21.6^{b}	1.25^{a}	2.59^{a}	2.98^{a}	1.40^{a}	0.33^{a}	115^{c}	2.6^{a}	8.2^{a}	138^{b}	22.6^{c}
Pooled std. dev.		1.5	1.1	0.05	0.7	3.2	0.2	3	0.3	0.6	0.4	0.2	0.08	1	0.2	0.3	10	0.8

Table 2
Chemical profile (mean, $n = 2$) of the virgin olive oils of Experiment 2 at different storage time (from T0 to T12 months). Phenolic compounds are reported in mg kg^{-1} of oil. Hydroxytyrosol (3,4-DHPEA); tyrosol (*p*-HPEA); hydroxytyrosol acetate (3,4-DHPEA Ac); decarboxymethyl oleuropein aglycone dialdehydic form (3,4-DHPEA-EDA); oleuropein aglycone dialdehydic form (DOA); decarboxymethyl ligstroside aglycone dialdehydic form (*p*-HPEA-EDA, oleocantal); ligstroside aglycone dialdehydic form (DLA); oleuropein aglycone (3,4-DHPEA-EA); ligstroside aglycone (LA). Sum of HPLC phenolics is the sum of phenolic concentration listed above. Free acidity as g free oleic acid kg^{-1} oil; peroxide index as meq O_2 kg^{-1} oil; oxidative stability in hours (induction time). Different letters in each column for each storage time represent significant differences ($p < 0,05$). The pooled standard deviation for each parameter is reported in the last line. MC_2, control (no preheating, 35 min malaxation); Pr_10′, 72 s preheating and 10 min malaxation.

Storage time	Sample Exper.2	3,4-DHPEA	*p*-HPEA	Vanillic acid	3,4-DHPEA Ac	3,4-DHPEA-EDA	DOA	Oleocantal	DLA	3,4-DHPEA-EA	LA	Luteolin	Apigenin	Sum of HPLC phenolics	Free acidity	Peroxide value	Total phenols	Oxidative stability
T0	MC_2	2.20^{a}	1.77^{a}	1.53^{a}	4.03^{a}	51.2^{a}	6.50^{a}	109^{a}	1.28^{a}	10.5^{a}	3.45^{a}	1.82^{a}	0.90^{a}	195^{a}	2.4^{a}	4.8^{a}	267^{a}	39.9^{a}
	Pr_10′	2.38^{b}	1.28^{b}	1.43^{a}	3.26^{a}	31.5^{b}	2.50^{b}	83.1^{b}	2.28^{b}	6.08^{a}	2.45^{b}	1.81^{a}	0.92^{a}	139^{b}	2.2^{a}	4.4^{a}	201^{b}	34.0^{b}
T3	MC_2	3.43^{a}	5.88^{a}	1.19^{a}	3.85^{a}	49.7^{a}	3.88^{a}	110^{a}	3.53^{a}	13.5^{a}	5.55^{b}	1.87^{a}	0.94^{a}	203^{a}	2.9^{b}	4.0^{a}	239^{a}	31.1^{a}
	Pr_10′	3.40^{b}	3.53^{b}	1.26^{a}	3.96^{a}	31.1^{b}	3.22^{b}	80.5^{b}	2.96^{b}	11.6^{a}	7.4^{a}	1.88^{a}	0.97^{a}	152^{b}	2.6^{a}	4.7^{a}	216^{a}	26.8^{b}
T6	MC_2	12.8^{a}	11.9^{a}	1.03^{a}	3.59^{a}	35.5^{a}	2.49^{b}	95.9^{a}	2.87^{a}	13.0^{a}	2.33^{b}	1.89^{a}	0.79^{a}	184^{a}	2.6^{a}	5.3^{b}	246^{a}	29.6^{a}
	Pr_10′	13.7^{b}	12.1^{a}	1.02^{a}	3.75^{a}	26.1^{b}	2.90^{a}	70.6^{b}	2.83^{a}	10.8^{b}	3.50^{a}	1.88^{a}	0.78^{a}	150^{b}	2.6^{a}	7.8^{a}	198^{b}	26.0^{b}
T12	MC_2	27.1^{a}	21.5^{a}	0.79^{a}	1.39^{a}	22.5^{a}	1.86^{a}	78.6^{a}	2.66^{a}	8.34^{a}	2.17^{a}	1.25^{a}	0.45^{a}	169^{a}	3.2^{a}	4.2^{b}	205^{a}	30.3^{a}
	Pr_10′	28.7^{a}	24.1^{a}	0.79^{a}	1.40^{a}	14.6^{a}	1.61^{a}	64.9^{b}	2.65^{a}	8.05^{a}	2.17^{a}	1.29^{a}	0.69^{a}	150^{b}	3.0^{a}	5.8^{a}	185^{a}	26.6^{b}
Pooled std. dev.		0.4	0.6	0.08	0.3	1.7	0.2	2.1	0.1	0.4	0.2	0.09	0.06	0.8	0.2	0.4	11	0.7

Table 3
Chemical profile (mean, $n = 2$) of the virgin olive oils of Experiment 3 at different storage time (from T0 to T12 months). Phenolic compounds are reported in mg kg^{-1} of oil. Hydroxytyrosol (3,4-DHPEA); tyrosol (*p*-HPEA); hydroxytyrosol acetate (3,4-DHPEA Ac); decarboxymethyl oleuropein aglycone dialdehydic form (3,4-DHPEA-EDA); oleuropein aglycone dialdehydic form (DOA); decarboxymethyl ligstroside aglycone dialdehydic form (*p*-HPEA-EDA, oleocantal); ligstroside aglycone dialdehydic form (DLA); oleuropein aglycone (3,4-DHPEA-EA); ligstroside aglycone (LA). Sum of HPLC phenolics is the sum of phenolic concentration listed above. Free acidity as g free oleic acid kg^{-1} oil; peroxide index as meq O_2 kg^{-1} oil; oxidative stability in hours (induction time). Different letters in each column for each storage time represent significant differences ($p < 0{,}05$). The pooled standard deviation for each parameter is reported in the last line. MC_3, control (no preheating, 35 min malaxation); Pf_35, 102 s preheating, no malaxation; Pf_50, 72 s preheating, no malaxation; Pf_75, 48 s preheating, no malaxation.

Storage time	Sample Exper.3	3,4-DHPEA	*p*-HPEA	Vanillic acid	3,4-DHPEA Ac	3,4-DHPEA-EDA	DOA	Oleocantal	DLA	3,4-DHPEA-EA	LA	Luteolin	Apigenin	Sum of HPLC phenolics	Free acidity	Peroxide value	Total phenols	Oxidative stability
T0	MC_3	0.68^{a}	0.94^{b}	1.21^{c}	3.53^{c}	138	3.78^{c}	88.8	1.13^{a}	7.99	3.16	2.08	0.98	252^{a}	2.0^{a}	4.0	321	42.6^{b}
	Pf_35	0.69^{a}	1.06^{a}	1.98^{a}	4.77^{ab}	143	4.12^{b}	89.0	0.97^{b}	7.56	3.15	2.07	0.99	259^{a}	2.0^{a}	4.0	318	43.8^{ab}
	Pf_50	0.25^{b}	0.44^{c}	1.64^{b}	4.90^{a}	154	4.65^{a}	91.1	0.88^{b}	7.79	3.11	2.08	1.0	272^{b}	1.8^{b}	3.6	328	43.9^{a}
	Pf_75	0^{c}	0.53^{c}	0.93^{c}	4.53^{b}	156	4.20^{a}	90.5	0.93^{b}	7.81	3.11	2.10	0.99	272^{b}	1.8^{b}	3.6	299	43.9^{a}
T3	MC_3	4.60^{a}	3.16^{a}	1.56^{a}	3.82^{b}	125	2.74^{a}	95.0	2.01^{c}	9.13^{ab}	2.59	2.08	1.05	253^{a}	2.7^{ab}	3.2	283	36.7^{b}
	Pf_35	3.10^{b}	1.60^{c}	1.66^{a}	4.91^{a}	132	1.47^{a}	98.1	2.01^{c}	7.98^{b}	2.51	2.11	1.07	259^{a}	2.8^{ab}	3.4	314	37.9^{ab}
	Pf_50	2.79^{c}	1.96^{b}	1.70^{a}	4.92^{a}	139	2.66^{a}	98.5	2.46^{b}	9.92^{a}	2.63	2.09	1.10	270^{b}	3.1^{a}	4.0	306	38.6^{a}
	Pf_75	2.10^{d}	1.28^{d}	1.73^{a}	4.57^{a}	132	2.66^{a}	99.4	2.80^{a}	8.79^{ab}	2.61	2.02	1.11	261^{ab}	2.3^{b}	4.0	300	37.3^{ab}
T6	MC_3	10.0	4.74^{a}	1.15^{a}	2.51^{c}	86.0	2.26^{b}	83.0	2.56^{b}	9.14^{b}	2.12^{b}	2.12	1.15	207^{a}	2.8^{a}	6.2^{bc}	252^{b}	33.0^{a}
	Pf_35	10.2	2.64^{d}	1.02^{b}	3.94^{b}	85.4	1.10^{c}	86.9	2.56^{b}	7.49^{d}	2.50^{a}	2.10	1.12	207^{a}	2.7^{a}	7.0^{ab}	277^{ab}	32.0^{a}
	Pf_50	10.1	3.50^{c}	1.15^{a}	5.11^{a}	87.6	2.71^{a}	87.6	3.26^{a}	9.42^{a}	2.29^{ab}	2.10	1.14	216^{b}	2.7^{a}	5.7^{c}	301^{a}	32.5^{a}
	Pf_75	10.9	4.09^{b}	1.23^{a}	5.16^{a}	85.9	2.53^{a}	89.2	2.63^{a}	8.53^{c}	2.18^{ab}	2.11	1.14	216^{b}	2.6^{a}	7.6^{a}	279^{ab}	32.0^{a}
T12	MC_3	26.8	19.5^{a}	0.98^{a}	2.86^{a}	75.1	1.87^{b}	69.8^{b}	2.40^{a}	7.46^{b}	2.09	1.67	0.83	211^{b}	2.6^{b}	3.1^{b}	308^{a}	35.6^{ab}
	Pf_35	29.5	20.7^{a}	0.13^{b}	2.08^{b}	69.0	1.16^{c}	68.4^{b}	1.32^{c}	6.06^{c}	2.17	1.64	0.89	203^{a}	2.8^{ab}	3.7^{ab}	259^{b}	36.5^{a}
	Pf_50	29.4	15.6^{b}	0.95^{a}	2.80^{a}	66.5	1.89^{b}	74.2^{a}	1.90^{b}	8.02^{a}	2.20	1.59	0.86	205^{a}	2.9^{ab}	4.3^{a}	319^{a}	36.4^{a}
	Pf_75	26.8	17.4^{c}	0.96^{a}	2.83^{a}	75.3	2.45^{a}	73.0^{a}	2.42^{a}	8.10^{a}	2.19	1.61	0.85	214^{b}	3.0^{a}	4.3^{a}	320^{a}	35.1^{b}
Pooled std. dev.		1.2	0.3	0.07	0.2	7.3	0.07	5.3	0.09	0.3	0.1	0.09	0.07	2.6	0.2	0.3	13	0.5

Experiment 1 (Pr and Pf) did not show peroxides significantly different from the control sample (MC_1). However, an increase of primary oxidation was observed at T0 and T12. In particular, after one year of storage, the peroxide content of sample Pf was 1.71 times that of the control, although the values were very low on average. Presumably, this increase might be related to the high temperature (43 °C) recorded after preheating the olive paste. In the further study period (T6 and T12) the experimental sample Pr_10 showed a peroxide number statistically higher than the control. The peroxide number was 1.47 and 1.38 times that of the control, at T6 and T12, respectively. At T0 and T3, the samples of the Experiment 3 showed a peroxide number very similar to the control sample. Samples collected at T6 and T12 were significantly different from the control but not to a big extent.

The sensory profile of the oils collected at T0, T3 and T12 are reported in Fig. 2 in the form of radar chart. Within the first three months, all the samples maintained the characteristics of the EVOO category. However, at T12, the samples Pr, Pf and Pf_35, had a defect score higher than 0 and thus they lost their attributes of EVOO and could be classified as VOOs. At T0, the samples of Experiment 1 (Pr and Pf) were characterized by a lower intensity of the bitter and pungency sensation and by a lower balance compared to the control sample. These differences were not seen after 3 months. After one year of storage, the samples Pr and Pf showed a reduction of the pungency sensation and showed the off-flavour of cooked vegetable, which is probably due to the high temperature reached during processing (42–43 °C). The sensory profile of the sample Pr_10′ during the storage was characterized by a slightly lower quality than the sample MC_2, even if they were well accepted, especially by those consumers appreciating the less bitter and pungency attributes of VOOs. Unlike the samples Pr and Pf, the Pr_10′ oil was not found to be defective after one year of storage. The oils of Experiment 3 showed high quality profile both at T0 and T3 and were similar to the control, except for the olfactory attributes (intensity of the olive fruitiness and green herbaceous

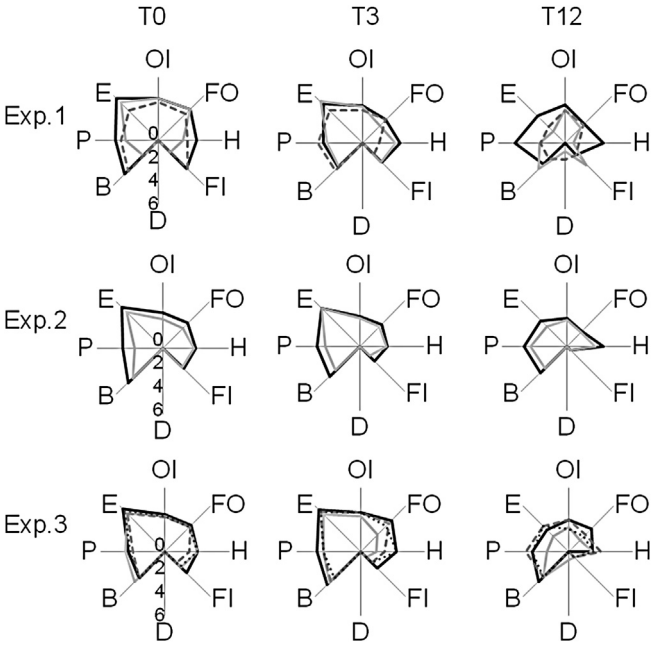

Fig. 2. Sensory profiles of ExtraVirgin Olive Oils evaluated in samples from the three experiments at T0, T3 and T12. OI, olfactory intensity; FO, fruity (of olive) intensity; H, intensity of herbaceous; FI, fruity intensity; D, defects; B, bitter; P, pungency; E, equilibrium. In Exp. 1, ▬: MC_1; ▬: Pr; ▬ ▬: Pf. In Exp.2, ▬: MC_2; ▬: Pr_10′. In Exp.3, ▬: MC_3; ▬: Pf_35; ▬ ▬: Pf_50; ▬ ▬: Pf_75.

sensation) of the sample Pf_35 at T3 that were considerably lower compared to the control. At T12, the Pf_50 oil appeared to be the spiciest and most herbaceous among all. The Pf_75 oil showed similar characteristics as the control, whereas Pf_35 was affected by a weak defect of 'cooked', as it happened with Experiment 1.

The phenolic content measured through the Folin–Ciocalteu reagent increased with the harvest date (Tables 1–3). The lowest phenolic content was observed in the oils of Experiment 1 (harvest date was November 3); then, the phenolic content increased gradually in Experiment 2 (November 25) and was highest in Experiment 3 (December 1st). Literature data show that oleuropein decreases with the maturation of the olives (Damak, Bouaziz, Ayadi, Sayadi, & Damak, 2008); however, more mature olives contain higher levels of dimethyloleuropein, hydroxytyrosol-4-β-glucoside, dimethylligstroside and oleoside-11-methyl ester, as well as glycosidic flavonoids such as luteolin-7-glucoside, quercetin-3-rutinoside (Esti, Cinquanta, & La Notte, 1998; Malik & Bradford, 2006) and cyanidin glycosides (Romero, Brenes, Garcia, & Garrido, 2002). The phenolic profile is closely related to the variety of olives, so each of them has its own optimum harvest date. Kukash (2010) observed that Frantoio and Leccino olives harvested in the second half of November and in the period between the second week of November and the first week of December, respectively, lead to the highest oil yield, as well as the highest oxidative stability and highest phenolic content. The VOO samples of Experiment 1 and 2 showed a lower content in phenols and a lower oxidative stability throughout the storage period compared to their control. For the samples Pr and Pf, the differences were statistically significant in almost all the monitored periods. The comparison showed a lower quality for Pf with respect to Pr; the major differences were observed at T12. In particular, the oxidative stability of Pf was 13% lower than Pr after a year of storage. Such evidence was caused by a lower content of phenolic substances (−30%). A significantly lower resistance to forced oxidation was found in the VOO belonging to Experiment 2 (12–15% lower at all storage periods) compared to their control. The phenolic content, although always lower than the control, exhibited statistically significant differences only at T0 and T6. The samples of Experiment 3 resulted to be very similar among them and with respect to the control sample in terms of oxidative stability and phenolic content, as it can be seen from Table 3. The sample produced using an intermediate time of preheating (Pf_50) showed a higher phenolic content compared to the other three samples (MC_3; Pf_35 and Pf_75) more frequently. The differences between Pf_50 and MC_3 at T6 were statistically significant whereas at other storage times they ranged 2–8%. Also the oxidative stability of Pf_50 was usually the highest among the samples. No significant differences existed between the control sample and Pf_35 as regards the oxidative stability and the phenolic content of the first storage periods. Only after a year of storage, the phenolic content of Pf_35 decreased significantly (−19%); however, this was not accompanied by a reduction of the oxidative stability compared to the control. The total phenolic content of the sample Pf_75 was the most similar to the control sample. As observed by other authors (Boselli et al., 2009; Clodoveo, Delcuratolo, Gomes, & Colelli, 2007), a positive correlation (R^2 = 0.71) between phenolic content and oxidative stability monitored throughout the experimental period was registered when considering all the samples. The variability of the correlation coefficient is related not only to the overall content of these substances but also to the phenolic profile, since each phenol shows different antioxidant activity. The components identified with HPLC were simple phenolic compounds (hydroxytyrosol, tyrosol, vanillic acid and hydroxytyrosol acetate), secoiridoids (decarboxymethyl oleuropein aglycone dialdehydic form, oleuropein aglycone dialdehydic form, decarboxymethyl ligstroside aglycone dialdehydic form, ligstroside aglycone dialdehydic form, oleuropein aglycone, ligstroside aglycone) and flavones (luteolin and apigenin) (Boselli, Di Lecce, Minardi, Pacetti, & Frega, 2007). Lignans were not found. In Tables 1–3 and in Fig. 3 are respectively reported the quantitative phenolic composition of all the samples and the evolution of the three classes of identified phenolics (simple phenolics, secoiridoids and flavones) during the storage. The absolute amount of phenolics estimated by using the Folin–Ciocalteu spectrophotometric method and chromatographic analysis were different although the trend outlined with both methods for each experiment was similar. The spectrophotometric method, as noted by several authors (Boselli et al., 2009; Escarpa &

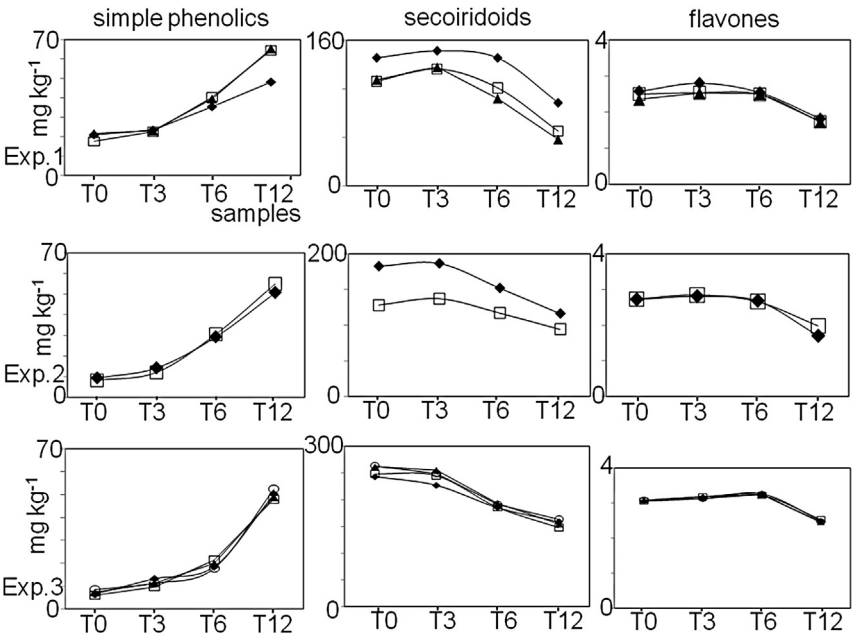

Fig. 3. Time evolution of simple phenolics, secoiridoids and flavones during the storage of ExtraVirgin Olive oils obtained from the three experiments. In Exp.1, —◆—: MC_1; —□—: Pr; —▲—: Pf. In Exp. 2, —◆—: MC_2; —□—: Pr_10′. In Exp.3, —◆—: MC_3; —□—: Pf_35; —▲—: Pf_50; —○—: Pf_75.

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González, 2001), estimated always a higher content of phenols with respect to HPLC. This discrepancy is due to interfering substances of non-phenolic nature which can contribute to the redox reaction. It should also be pointed out that some minor peaks present in the chromatograms could not be identified and were therefore not included in the HPLC quantification. In general, the trends shown by the different phenolic classes was similar for all samples. Within the first three months of storage, tyrosol and hydroxytyrosol were scarcely present. Then, a significant increase of these compounds was registered in the following nine months of storage. This increase was associated with the process of lysis of secoiridoids, mainly affecting 3,4-DHPEA-EDA and p-HPEA-EDA which are respectively precursors of 3,4-DHPEA and p-HPEA. In fact, decarboxymethylation is the first reaction affecting secoiridoids during the storage of VOO as previously reported by the same authors. Subsequently, lysis processes determine the break of the ester bond between elenolic acid and tyrosol or hydroxytyrosol, causing an increase of the latter, by the third month onwards (Boselli et al., 2009). Consequently, a decrease of secoiridoids occurred together with the increase of simple phenols. Secoiridoids were predominant up to T3, but then decreased in the following nine months of storage. As regards the flavones luteolin and apigenin, their concentration was roughly constant until T6 and then decreased in the following six months, presumably due to oil oxidation. During the storage, the phenolic species observed in the experimental samples followed a similar trend to that observed in the control. However, in the same way to what resulted from the Folin–Ciocalteu procedure, the VOOs of Experiment 1 and 2 had always a lower amount of phenolic compounds compared to the respective control (Tables 1 and 2). In particular, the differences were mainly due to the complex phenolic compounds being always lower in the Experiment 1 and 2. These differences already appeared at T0. However, Pr_10′ showed a lower secoiridoid content all through the storage period, whereas Pr and Pf showed evident discrepancies in the 3,4-DHPEA-EDA content. At T12, however, such differences interested all the secoiridoids indiscriminately. Parallel to these decrease, a higher content of 3,4-DHPEA and p-HPEA were recorded in Pr, Pf and Pr_10′, thus confirming the big extent of lysis of the corresponding secoiridoid species. Compared to the samples of Experiment 1 and 2, those of Experiment 3 were characterized by a higher content of phenolic compounds (Table 3). This was in agreement with the spectrophotometric findings. Secoiridoids were present at higher levels than the relative control during the storage, but in general the differences were not significant. During the whole period of storage the samples showed very similar profiles to each other and to the MC_3 oil.

4. Conclusions

From the results it can be observed that fast preheating of the olive paste can replace malaxation under certain conditions of time and temperature. However, the processing temperature is a fundamental parameter in determining the quality of VOO, evaluated both in the fresh oil and during the first year of storage. The processing temperature strongly influences the activity of PPO and POD and thus the phenolic composition of the resulting virgin olive oil not only during malaxation (Taticchi et al., 2013), but also during the preheating process. For this reason, we decided to monitor the quality of the VOO from preheated olive paste during the storage of the oil, unlike previous research work (Esposto et al., 2013). Although there are no prescriptions on the temperature requirements during olive processing (temperature requirements are only specified in the EU Reg. 29/2012, as an optional indication for cold pressing/extraction), virgin olive oils must comply with the quality characteristics set out in the Annex I to the EEC Regulation No 2568/91. So, temperatures around 42–43 °C during preheating were deleterious for the VOO quality compared to 32 °C, because the oils showed a lower phenolic content and lower oxidative stability. The decline of the EVOO quality at these temperatures became already evident in the first few months of storage and increased after one-year storage. In fact, at T12, the sensory panel recorded the defect of 'cooked' in these oils. Instead, preheating temperatures of 37–38 °C determined different effects on the final quality of the oils depending on the presence or absence of malaxation. As a matter of fact, the phenolic content decreased in the oil when fast preheating was followed by malaxation.

As a general rule, two processing options can be prospected from the present data; a) a fast preheating not longer than 72 s at 38 °C (without malaxation) lead to a 'robust' EVOO (sometimes with a higher phenolic content, as in the samples Pf_50 and Pf_75) with a minimum 12-months shelf life; b) a fast preheating not longer than 72 s at 38 °C followed by 10 min malaxation lead to a virgin olive oil with a mild sensory profile and a 12-months shelf-lfe without sensory defects.

Olive pastes preheated for a longer time (102 s) at 38 °C lead to a virgin olive oil with an off-flavour (cooked) arising in the T12 sample. In other words, the use of a preheater at less than 38 °C may lead to a quality advantage, which is related to the modulation of the phenolic content. As stated above, the oil temperature did not exceed 42 °C (measured value) for 102 s during our experimental work, thus the waxes content should not exceed the legal limit as reported elsewhere (Di Giovacchino et al., 2002). The adjustment of the transit time and temperature in the fast preheater is crucial to achieve the desired final quality of the EVOO. The temperature of the olive paste can be tuned according to the quality and phenolic content of the fresh olives and of the desired characteristics of the resulting EVOO. In fact, with fast preheating followed by a short malaxation (e.g. 10 min), a EVOO low in phenolics can be obtained from olives with a high phenolic content for consumers who prefer less bitter and pungent EVOOs. The application of preheating can be interesting for non mediterranean markets, or in the case of olive batches with an unpleasant high phenolic content, or to obtain a 'mild' EVOO already at T0 without a maturation time, or when olives were harvested on a too early date. In addition, this is a desirable strategy in an olive oil mill where the daily working capacity can be optimized thanks to the faster processing time avoiding the usual 30–45 min of malaxation.

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