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Flash Thermal Conditioning of Olive Pastes during the Olive Oil Mechanical Extraction Process: Impact on the Structural Modifications of Pastes and Oil Quality

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ABSTRACT: The quality of virgin olive oil (VOO) is strictly related to the concentrations of phenolic and volatile compounds, which are strongly affected by the operative conditions of the VOO mechanical extraction process. The aim of this work is to study the impact of a new technology such as flash thermal conditioning (FTC) on olive paste structural modification and on VOO quality. The evaluation of olive paste structure modification by cryo-scanning electron microscopy (cryo-SEM) showed that the application of FTC after crushing produces significant differences in terms of the breaking of the parenchyma cells and aggregation of oil droplets in comparison to the crushed pastes. The virgin olive oil flash thermal conditioning (VOO-FTC) featured a higher concentration of volatile compounds compared to that in the control, particularly of all saturated and unsaturated aldehydes and esters, whereas the phenolic concentration was higher in VOO obtained from the traditional process (VOO-C).

KEYWORDS: *virgin olive oil, flash thermal conditioning, cryo-SEM, phenols, volatile compounds*

INTRODUCTION

Virgin olive oil (VOO) is one of the main components of the Mediterranean diet thanks to its particular chemical composition. Nowadays, the minor components, more than the well-known fatty acid composition characterized by a high nutritional value, make the olive oil a fatty substance with unique properties. The minor components, which represent approximately 2% of oil weight, are over 230 chemical substances belonging to different classes such as aliphatic and triterpene alcohols, sterols, hydrocarbons, volatile compounds, and polyphenols. The most important regarding the health and sensory quality of virgin olive oil are polyphenols and volatile compounds.

The health properties of VOO are related to the fatty acid composition, tocopherols, and hydrophilic phenols such as phenolic acids, phenolic alcohols [3,4-dihydroxyphenylethanol (3,4-DHPEA), and *p*-hydroxyphenylethanol (*p*-HPEA)], and aglycone derivatives of secoiridoids [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].[1−5] These compounds are also responsible for the oxidative stability of VOO[6−11] and for the bitter and pungent gustative notes, while some volatile compounds are correlated to its aroma.[12] The C_5 and C_6 saturated and unsaturated aldehydes and corresponding alcohols, in particular, were identified as responsible for the cut grass and green olfactory notes, whereas the esters were associated with the fruity sensations. These volatile compounds were originated by the lipoxygenase (LOX) pathway activated during the oil's mechanical extraction process.[12−15] The olive oil mechanical extraction process includes three main steps: olives crushing, malaxation, and separation of the oil phase by pressure or centrifugation. Crushing and malaxation are the processing steps more directly involved in the phenolic release and aroma generation in VOO.[5,16−18]

The first aim of malaxation is the aggregation of small oil droplets, dispersed in the olive pastes, to facilitate oil separation during the mechanical extraction process. So far, however, the control of time, temperature, and oxygen availability during processing strongly affects the phenolic and volatile composition of VOO.[4,19−25]

The traditional malaxation process performs paste mixing and thermal conditioning at the same time, but the thermal transfer efficiency is generally low, and for this reason, the thermal conditioning of olive pastes is relatively long compared to the optimal processing temperature. This aspect influences the activity involved in oil extraction of the endogenous enzymes, such as pectinases, hemicellulases, and cellulases, as well as the polyphenoloxidase (PPO), peroxidase (POD), and LOX that affect the phenolic and volatile composition of VOO. The fast heating technology applied to the thermal conditioning of the olive pastes before malaxation is a new approach to the oil mechanical extraction process, which can revise the traditional thermal conditioning applied to the olive pastes during the oil's mechanical extraction process. The flash heating of paste after crushing can eliminate the malaxation

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time required for thermal conditioning with significant impact on the plant's working capacity, oil yield, and VOO quality.

This work investigates the effects of flash thermal conditioning (FTC) on the structure modifications of olive pastes and VOO volatile and phenolic composition.

MATERIALS AND METHODS

VOO Mechanical Extraction Process. VOO was extracted from olives of *Peranzana* and *Gentile* cultivars. The olives were processed in an industrial plant belonging to the oil mill, Frantoio Gonnelli "Santa Tea" – Reggello (FI, Italy).

The control trial for the production of the virgin olive oil control (VOO-C) was conducted using traditional, Alfa Laval (Tavarnelle V.P. Florence, Italy) plant technology with an average process capacity of 2.5 t/h. The olive mill consisted of a disc crusher, traditional covered malaxer, three-phase decanter extractor NX X30 ECB/VS, and double vertical separator UVPX507 for the oily phase recovery and for its separation from the vegetation water. The traditional malaxation was carried out for 40 min at 25 or 30 °C.

After the same disc crusher was used for control, the flash thermal conditioning of olive pastes was obtained using an EVO-Line heat exchanger from Alfa Laval with 2.5 t/h of capacity, which was heated by hot water at 35 and 40 °C to bring the pastes at 25 or 30 °C, respectively.

The paste was heated instantaneously using the EVO-Line at those fixed temperatures. Heating was carried out with counter current, hot water flow, automatically regulated by specifically regulated valves controlled by a programmable logic controller (PLC). Heated paste was sent to a sealed paste malaxer, and the oil was extracted using the same decanter and vertical separator used for the control (Figure 1).

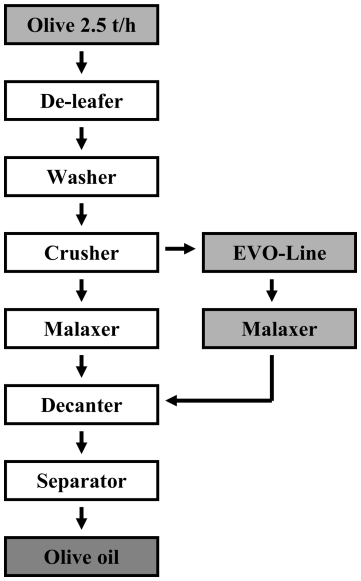

Figure 1. Flow-chart of the virgin olive oil mechanical extraction process, traditional and flash thermal conditioning (EVO-Line).

The trials were performed to test 2 flash conditioning temperatures (25 and 30 °C) and 3 different malaxation times (0, 5, 10 min). These times were counted since the complete filling of the standstill malaxer. VOO samples were taken after 15 min from the beginning of separation of the oil phase and stored in the dark at 13 °C until analysis. Samples of pomace were stored at −25 °C until analysis.

Reference Compounds. (3,4-Dihydroxyphenyl)ethanol (3,4-DHPEA) was obtained from Cayman Chemicals Ltd. (United States), whereas the (*p*-hydroxyphenyl)ethanol (*p*-HPEA) was obtained from Janssen Chemical Co. (Beerse, Belgium). The dialdehydic forms of elenolic acid linked to 3,4-DHPEA and *p*-HPEA (3,4-DHPEA-EDA and *p*-HPEA-EDA, respectively), the isomer of oleuropein aglycon (3,4-DHPEA-EA), the (+)-1-acetoxypinoresinol, and (+)-pinoresinol were extracted from VOO using a procedure previously reported.[1] In short, the phenols were extracted from the oil (1 kg) using methanol/water 80:20 v/v (2 L). The moisture oil (1 kg)/methanol–water (500 mL) was homogenized for 3 min at 25 °C. Then, it was centrifuged at 3000 rpm for 20 min. After the recovery of the supernatant, this procedure was repeated three times. After solvent evaporation and partial purification of the crude extract obtained from the olive fruit and VOO, the phenolic separation was performed by semipreparative high-performance liquid chromatography (HPLC) analysis using a 9.4 mm ID × 500 mm Whatman Partisil 10 ODS-2 semipreparative column; the mobile phase was 0.2% acetic acid in water (pH 3.1) (A)/methanol (B) at a flow rate of 6.5 mL/min, whereas the phenol detection was performed using a diode array detector (DAD).[1] The purity of all the substances obtained from direct extraction was tested by HPLC, and their chemical structures were verified by nuclear magnetic resonance (NMR) using the same operative conditions reported in previous papers.[1,26] Pure analytical standards of volatile compounds were purchased from Fluka and Aldrich (Milan, Italy).

Structure Modifications of Olive Pastes by Cryo-SEM. The structural modification of olive pastes after crushing and after malaxation was investigated for both the traditional and FTC systems. Samples of pastes were taken after crushing and after malaxation and immediately frozen quickly in liquid nitrogen (−196 °C), where they were stored in a frozen–hydrated (FH) state until the analysis by Cryo-SEM. For the analysis of the texture, the FH samples of pastes were first mounted under liquid nitrogen gas in an aluminum stub with Tissue-Tek, freeze-fractured inside liquid nitrogen to expose the internal texture, transferred to a dedicated cryo-preparation chamber (SEM Cryo Unit, SCU 020, Bal-Tech, Balzers, Liechtenstein), surface etched for 3 min at −80 °C under high vacuum (pressure < 2×10^{-4} Pa), and sputter-coated with 8 nm of gold in an argon atmosphere (pressure < 2.2×10^{-2} Pa) to produce an electrically conductive surface. FH specimens were finally transferred to the cryo-stage (−180 °C) inside a scanning electron microscope (Philips SEM 515, Eindhoven, The Netherlands).[27]

VOO Analyses. *Marketable Parameters.* The free acidity and peroxide values were measured in accordance with the European Official Methods.[28]

Oil Content. Foss-Let 15310 (A/S N. Foss Electric Denmark) was utilized for the quantitative evaluation of pomace oil content. Dry pomace (22.5 g) was homogenized (Homogenizer, A/S N. Foss Electric Denmark) with 120 mL of tetrachloroethylene with the addition of a small amount of anhydrous sodium sulfate for 2 min and estimated.

Phenolic Compounds. The analysis of VOO was conducted with direct injection dissolving 1 g of oil in 5 mL of acetone, and then the solution was filtered through a polyvinylidene fluoride (PVDF) syringe filter (0.2 μm). As described by Selvaggini et al.,[29] HPLC analysis was performed using an Agilent Technologies system model 1100 consisting of a vacuum degasser, a quaternary pump, an autosampler, a thermostatted column compartment, a diode array detector (DAD), and a fluorescence detector (FLD), controlled by ChemStation (Agilent Technologies, Palo Alto, CA. USA), and used for the elaboration of chromatographic data. A Spherisorb ODS-1 column was used to evaluate the phenolic compounds, the mobile phase consisted of 0.2% acetic acid (pH 3.1) in water (solvent A)/methanol (solvent B) at a flow rate of 1 mL/min, and the gradient changed as follows: 95% A for 2 min, 75% A in 8 min, 60% A in 10 min, 50% A in 16 min, and 0% A in 14 min and maintained for 10 min. Following the re-equilibration of the initial conditions, equilibration was reached in 13 min; the total running time was 73 min. All phenolic compounds, except lignans, which were detected by FLD, operated at an excitation wavelength of 280 nm and emission at 339 nm,[30] were detected by DAD at 278 nm.

Volatile Compounds. Evaluation and quantification of volatile compounds in VOOs were performed by headspace and solid-phase microextraction, followed by gas chromatography–mass spectrometry (HS-SPME/GC-MS) according to Servili et al.[31] Six grams of oil with the addition of 50 μL of a standard methanolic solution consisting of

Table 1. Moisture and Oil Content of Pomaces of cv. *Peranzana* Processed at 30 °C for 0, 5, and 10 min of Malaxation Time[a]

	cv. *Peranzana*			
	control	FTC (0 min)	FTC (5 min)	FTC (10 min)
moisture content (%)[b]	51.2 (0.6) a	53.0 (0.4) b	53.5 (0.4) b	53.2 (0.4) b
oil content (% d.w.)	8.9 (1.0) a	11.3 (0.5) b	10.9 (0.6) b	10.3 (0.5) ab

[a]FTC = flash thermal conditioning. [b]Data are the mean values of two independent experiments. The values in each row with different letters (a,b) are significantly different from one another ($p < 0.05$). The mean values of olive moisture and oil content are, respectively, 58.1% and 37.4% d.w.

Table 2. Moisture and Oil Content of Pomaces of cv. *Gentile* Processed at 25 °C and 30 °C for 0 min of Malaxation Time[a]

	control (25 °C)	FTC (25 °C)	control (30 °C)	FTC (30 °C)
moisture content (%)[b]	52.6 (0.6) a	54 (0.8) a	53.8 (0.6) a	53.2 (0.6) a
oil content (% d.w.)	10.6 (1.0) a	12.1 (0.4) b	11.5 (0.1) ab	11.8 (0.1) ab

[a]FTC = flash thermal conditioning. [b]Data are the mean values of two independent experiments. The values in each row with different letters (a,b) are significantly different from one another ($p < 0.05$). The mean values of olive moisture and oil content are, respectively, 58.8% and 41.6% d.w.

butanal, isobutyl acetate, and 1-nonanol was mixed with vortexing for 1 min, and then 3 g was placed into a 10-mL vial. For the sampling of the headspace volatile compounds, solid-phase microextraction (SPME) was applied as follows: all the vials were held at 35 °C, and then, the SPME fiber (a 50/30 μm DVB/Carboxen/PDMS 1 cm in length, Stableflex; Supelco, Inc., Bellefonte, PA) was exposed to the vapor phase for 30 min to sample the volatile compounds. Afterward, the fiber was inserted into the gas chromatograph (GC) injector, set in splitless mode, using a splitless inlet liner of 0.75 mm ID for thermal desorption, where it was left for 10 min. All of the SPME operations were automated through the Varian CP 8410 Autoinjector (Varian, Walnut Creek, CA).

GC-MS Analysis. A Varian 4000 GC-MS equipped with a 1079 split/splitless injector (Varian, Walnut Creek, CA) was used. A fused-silica capillary column was employed (DB-Wax-ETR, 50 m, 0.32 mm ID, 1 μm film thickness; J&W Scientific, Folsom, CA). The column was operated with helium at a constant flow rate of 1.7 mL/min, maintained with an electronic flow controller (EFC). The GC oven heating program started at 35 °C. This temperature was maintained for 8 min, then increased to 45 °C at a rate of 1.5 °C/min, increased to 150 °C at a rate of 3 °C/min, increased to 180 °C at a rate of 4 °C/min, and finally increased to 210 °C at a rate of 3.6 °C/min; this temperature was held for 14.5 min. The total analysis time was 80 min. The injector temperature was maintained at 250 °C, the temperature for the transfer line was fixed at 170 °C, and the mass spectrometer was operated in the electron−ionization (EI) mode at an ionization energy of 70 eV, with scanning in the mass range of m/z 25−350 at a scan rate of 0.79 s/scan and a manifold temperature of 150 °C. The GC-MS was operated with Varian MS Workstation Software, version 6.6. The volatile compounds were identified by comparison of their mass spectra and retention times with those of authentic reference compounds. Integration of all the chromatographic peaks was performed by choosing the three masses with the highest intensities from among those specific for each compound, to selectively discriminate them from their nearest neighbors. The results of the peak areas were calculated on the basis of the relative calibration curve for each compound and expressed in μg/kg of oil.[31]

RESULTS AND DISCUSSION

Oil Separation Efficiency. The use of FTC in olive pastes during the oil's mechanical extraction process reduced the oil's separation efficiency compared to the traditional process. However, the differences of the residual oils in the pomaces obtained after FTC treatment compared to the control were lower, increasing the malaxation time and the processing temperature of pastes after FTC treatment. In fact, as reported in Table 1, the variations of the residual pomace oil compared to those of the control were 2.4, 2, and 1.45% when the FTC treatment was followed by 0, 5, and 10 min of malaxation at 30 °C, in *Peranzana* cv. Furthermore, the oil content of the two pomaces (FTC and control) processed at 30 °C for 0 min was not significantly different (11.45% and 11.8% for the control and FTC, respectively) in *Gentile* cv. (Table 2).

Cryo-SEM Analysis. The results in terms of oil yield obtained using the FTC treatment after crushing enabled the conclusion to be made that flash thermal treatment of olive paste produces a structural modification of pastes involving the aggregation of oil drops, which is critical to obtain an efficient oil separation by centrifugation. Cryo-SEM analysis of the structural characteristics of olive pastes after olive crushing (Figure 2) and after malaxation (Figure 3) allowed the paste's microstructure characteristics to be investigated both in the traditional (Figures 2A and 3A) and FTC (Figures 2B and 3B) systems. In terms of the breaking down of olive parenchyma cells and the aggregation of oil droplets, density appears to confirm that stated above. The cryo-SEM images show that, after the crushing of the olives (Figure 2A), the pastes still contain several whole parenchyma cells, with intact cell wall and oil droplets inside the vacuole. On the contrary, in the pastes obtained after crushing and subsequent FTC treatment (Figure 2B), the cell walls are completely destroyed, and the small droplets of oil can leak from vacuoles and aggregate between them. However, at this point in the pastes obtained using FTC system, the size of just oil droplet aggregates also does not exceed the diameter of 13.2 ± 5.2 μm. At the end of malaxation, the cryo-SEM images show that by using the traditional system (Figure 3A), the paste contains a few drops of oil aggregates. However, there are still some very small droplets (22.2 ± 17.3 μm). Similarly, at the end of malaxation using the FTC system, the oil is aggregated in both large and small drops (Figure 3B). The cryo-SEM study of the structural modification of olive pastes in the oil mechanical extraction process demonstrates that, in terms of disruption of the parenchyma cells and aggregation of oil droplets, the use of the FTC system after crushing produces a structural modification of the pastes, which can be considered to be similar to that caused by the traditional malaxation system.

VOO Qualitative Evaluation. *Marketable Parameters.* From a qualitative point of view, no significant differences were found for the marketable parameters free acidity and peroxide values (Tables 3 and 4).

Phenolic Composition. In both the cultivars studied, the VOO-FTC showed a lower phenolic concentration compared to the VOO obtained by the traditional process. The most important modifications, observed in the *Gentile* cv., were at 30

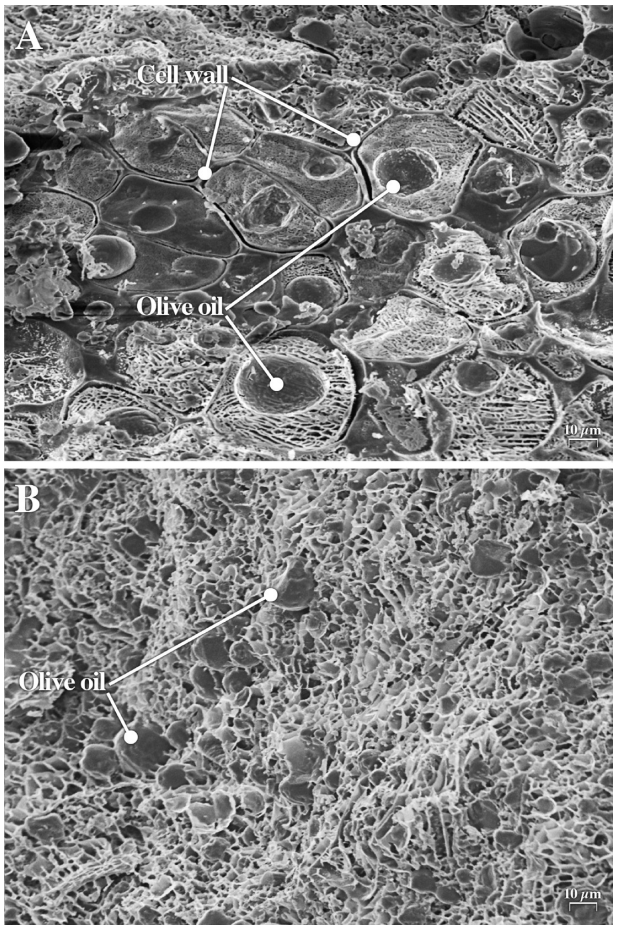

Figure 2. Cryo-SEM images of frozen-hydrated freeze-fractured samples of olive pastes of cv. *Peranzana* after crushing (A) and after FTC (B). In A, are yet visible several intact parenchyma cells, with cell wall and oil inside vacuoles, while in B, cell walls are completely destroyed, and the small oil droplets begin to aggregate.

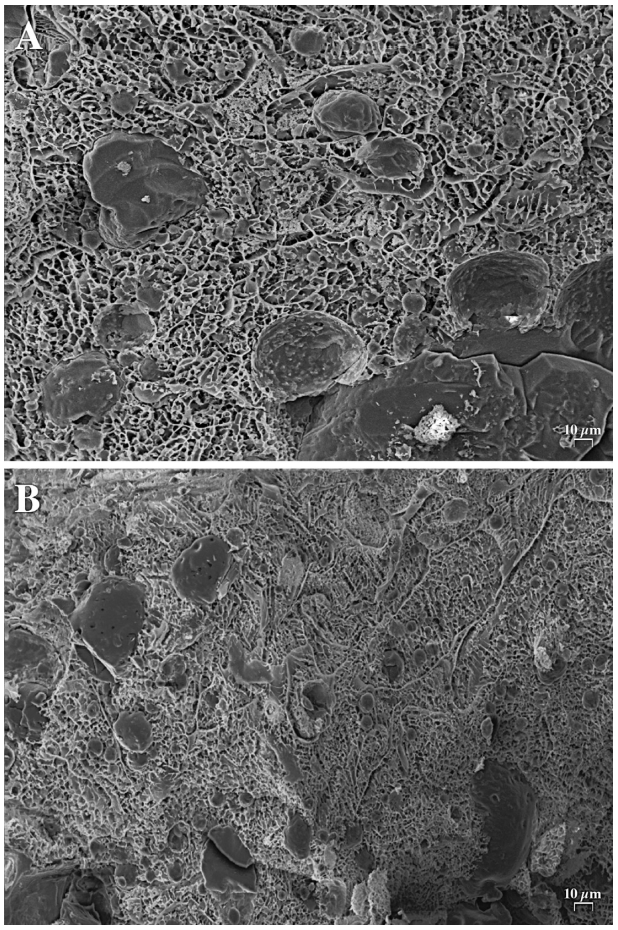

Figure 3. Cryo-SEM images of frozen-hydrated freeze-fractured samples of olive pastes of cv. *Peranzana* after malaxation in the traditional system (A) and in the FTC system after 10 min of malaxation time (B). In A and in B, the oil droplets are aggregated, but there are still many small droplets.

°C, where the sum of the phenolic fractions of the VOO-FTC decreased of 81.7 mg/kg with respect to the VOO-C (Table 5). The secoiridoids derivatives, which had higher reductions, were 3,4 DHPEA-EDA and 3,4 DHPEA-EA, whereas the lignans did not show any significant variations. However, these losses were reduced, increasing the malaxation time after FTC treatment (Table 6). In fact, as observed in *Peranzana* cv., malaxing the FTC pastes at 30 °C for 0, 5, or 10 min resulted in lower phenol content (expressed as sum) in the relative VOOs, respectively, of 19.4, 15.1, and 8.1% than that of VOO-C (Table 6). Those results can be explained by considering differences in terms of the activation period of depolymerizing enzymes between the FTC and the traditional process. As reported in previous papers, in fact the endogenous pectinases, hemicellulases, and cellulases, hydrolyzing the cell wall, improve the amount of phenolic compounds released in the oil and vegetation water during processing.[32,33]

In this context, the time and temperature of malaxation show a strong impact.[5] The oil extraction from the olive pastes, performed immediately after the FTC process, reduced the time for cell wall hydrolytic degradation, catalyzed by the depolymerizing enzymes and, as a consequence, the corresponding release of phenolic compounds in the oil (Table 5). For that reason, we could appreciate a significant improvement of phenolic concentration in the oil according to the malaxation time after FTC treatment (Table 6).

Volatile Composition. All of the VOOs-FTC showed significant modifications in terms of volatile compounds, due to the LOX pathway during processing, compared with the respective controls.

With regard to the trial carried out at different temperatures of malaxation on *Gentile* cv. olives, the VOO-FTC demonstrated a significant increase in (*E*)-2-hexenal, a reduction of C_6 saturated and unsaturated alcohols, and a slight increase in C_5 unsaturated alcohols (Table 7) compared to the head spaces of the VOOs obtained by malaxing the pastes with the traditional process (at 25 or 30 °C for 40 min).

However, the values obtained showed that the oils extracted at 25 °C were characterized by a greater concentration of saturated and unsaturated aldehydes, whereas C_6 and C_5 saturated and unsaturated alcohols and esters remained almost unchanged compared to those in the trials processed at 30 °C (Table 7).

Compared to the VOO-C, (*E*)-2-hexenal increased 32.4% and 12% in the FTC-VOO head space when the pastes were malaxed, respectively, at 25 and 30 °C. Even the esters showed higher values in VOOs-FTC than those obtained by the traditional process. In that case, the augmentation for hexyl-acetate was of 56.2% and 62.8% malaxing the FTC pastes at 25

Table 3. Free Acidity and Peroxide Values of VOO-C and VOO-FTC Obtained by Processing cv. *Peranzana* at 30 °C for 0, 5, and 10 min of Malaxation Time[a]

	VOO-C	VOO-FTC (0 min)	VOO-FTC (5 min)	VOO-FTC (10 min)
acidity (g of oleic acid/100 g of oil)[b]	0.28 (0.01) a	0.29 (0.02) a	0.32 (0.01) a	0.29 (0.01) a
peroxide value (meq of O_2/kg of oil)	10.20 (0.67) a	9.73 (0.98) a	9.52 (1.09) a	9.55 (0.84) a

[a]VOO-C = virgin olive oil control; VOO-FTC = virgin olive oil flash thermal conditioning. [b]Data are the mean values of two independent experiments. The values in each row with different letters (a,b) are significantly different from one another ($p < 0.05$).

Table 4. Free Acidity and Peroxide Values of VOO-C and VOO-FTC Obtained by Processing cv. *Gentile* at 25 and 30 °C for 0 min of Malaxation Time[a]

	VOO-C (25 °C)	VOO-FTC (25 °C)	VOO-C (30 °C)	VOO-FTC (30 °C)
acidity (g of oleic acid/100 g of oil)[b]	0.34 (0.01) a	0.34 (0.01) a	0.34 (0.01) a	0.31 (0.01) a
peroxide value (meq of O_2/kg of oil)	9.52 (1.01) a	9.25 (1.12) a	9.93 (0.89) a	10.34 (0.73) a

[a]VOO-C = virgin olive oil control; VOO-FTC = virgin olive oil flash thermal conditioning. [b]Data are the mean values of two independent experiments. The values in each row with different letters (a,b) are significantly different from one another ($p < 0.05$).

Table 5. Phenolic Composition (mg/kg) of VOO-C and VOO-FTC Extracted at 25 and 30 °C for 0 min of Malaxation Time Extracted from Olives of cv. *Gentile*

compd	VOO-C (25 °C)	VOO-FTC (25 °C)	VOO-C (30 °C)	VOO-FTC (30 °C)
3,4-DHPEA[a]	6.0 (0.0) a	1.2 (0.1) b	1.6 (0.1) c	0.6 (0.0) d
p-HPEA	5.3 (0.1) a	3.8 (0.2) b	2.4 (0.1) c	2.4 (0.0) c
3,4-DHPEA-EDA	182.8 (5.8) ac	148.5 (9.4) b	204.7 (5.5) c	166.0 (18.1) ab
p-HPEA-EDA	61.4 (1.5) ab	55.7 (4.1) b	71.6 (3.5) c	66.2 (1.4) ac
ligstroside aglycon	9.8 (0.2) a	7.0 (0.03) b	10.3 (0.2) a	8.8 (0.3) c
3,4-DHPEA-EA	103.7 (0.6) a	104.8 (2.3) a	146.4 (0.9) b	110.0 (1.8) c
(+)-1-acetoxypinoresinol	12.0 (0.7) a	12.5 (0.5) a	12.7 (0.6) a	12.2 (0.4) a
(+)-pinoresinol	23.1 (1.1) a	24.1 (1.3) a	21.4 (1.2) a	23.2 (1.8) a
sum of phenolic fractions	404.1 (6.0) a	357.7 (10.5) b	471.2 (6.6) c	389.5 (18.2) a

[a]The data are the mean values of two independent experiments analyzed in duplicate, and the standard deviation is reported in parentheses. The values in each row with different letters (a–d) are significantly different from one another ($p < 0.05$).

Table 6. Phenolic Composition (mg/kg) of VOO-C and VOO-FTC Extracted at 30 °C for 0, 5, and 10 min of Malaxation Time Extracted from Olives of cv. *Peranzana*

compd	VOO-C	VOO-FTC (0 min)	VOO-FTC (5 min)	VOO-FTC (10 min)
3,4-DHPEA[a]	3.1 (0.1) a	2.9 (0.1) a	2.3 (0.1) b	3.1 (0.1) a
p-HPEA	3.2 (0.1) a	4.6 (0.1) b	4.2 (0.5) b	1.3 (0.2) c
3,4-DHPEA-EDA	288.7 (18.3) a	207.8 (10.9) b	216.0 (8.2) b	272.8 (15.0) a
p-HPEA-EDA	48.9 (4.1) a	47.3 (1.1) ab	42.6 (0.8) bc	40.0 (0.04) c
ligstroside aglycon	10.0 (0.4) a	10.2 (0.2) a	10.8 (0.2) a	12.0 (0.7) b
3,4-DHPEA-EA	111.1 (2.3) a	98.7 (6.1) ab	113.5 (6.2) a	96.3 (9.2) b
(+)-1-acetoxypinoresinol	15.0 (1.1) a	13.5 (0.9) a	14.9 (0.7) a	13.2 (1.0) a
(+)-pinoresinol	32.6 (2.7) a	28.0 (1.2) a	30.8 (1.9) a	32.6 (2.2) a
sum of phenolic fractions	512.6 (19.1) a	413.0 (12.5) b	435.1 (10.3) bc	471.3 (17.6) c

[a]The data are the mean values of two independent experiments analyzed in duplicate, and the standard deviation is reported in parentheses. The values in each row with different letters (a–d) are significantly different from one another ($p < 0.05$).

and 30 °C, respectively, while for (*Z*)-3-hexenyl acetate, the increase was of 61.5% (at 25 °C) and of 63.4% (at 30 °C).

The results on VOO-FTC of the *Peranzana* cultivar, malaxed at 30 °C at 0 min, showed the same trend observed in *Gentile* cv. VOOs both for the unsaturated aldehydes and esters showing higher increases for C_5 unsaturated alcohols, whereas C_6 saturated and unsaturated alcohols demonstrated an opposite trend (Table 8).

These results allowed us to observe the variability according to the cultivar in the generation of volatile compounds during processing, due to the different activity levels of each enzyme involved in the LOX pathway.[13]

Furthermore, it was possible to assume that the increase in the accumulation of C_6 saturated and unsaturated aldehydes observed in VOO-FTC in both the cultivars studied was due to the thermal stability of hydroperoxide lyase (HPL). As reported by Salas and Sánchez,[34] the optimal activity temperature for HPL was 15 °C, whereas the enzyme shows partial inactivation at 30 °C. The short time needed for the pastes treated with FTC to reach 25 °C and 30 °C compared to that in the traditional process, together with the corresponding elimination of the stop period at the maximum temperature, could reduce partial inactivation of HPL, promoting the higher accumulation of C_6 saturated and unsaturated aldehydes in VOO-FTC. In fact, all the VOOs-FTC extracted after 0 min of malaxation, showed a higher amount of C_6 saturated and unsaturated aldehydes compared to the control at the same temperature.

Table 7. Volatile Composition (μg/kg) of VOO-C and VOO-FTC Extracted at 25 and 30 °C for 0 min of Malaxation Time Extracted from Olives of cv. *Gentile*

compd	VOO-C (25 °C)	VOO-FTC (25 °C)	VOO-C (30 °C)	VOO-FTC (30 °C)
		Aldehydes		
hexanal[a]	449 (24.0) a	521 (16.9) b	424 (2.8) a	384 (8.2) c
(*E*)-2-pentenal	57 (1.4) a	60 (1.3) a	70 (4.2) b	62 (0.6) a
(*E*)-2-hexenal	27030 (438.4) a	40019 (598) b	32195 (63.6) c	36568 (335.7) d
		Alcohols		
1-penten-3-ol	335 (4.2) a	445 (3.9) b	425 (9.2) c	439 (3.2) b
1-pentanol	51 (4.2) a	30 (2.0) bc	33 (0.7) b	25 (1.9) c
(*E*)-2-penten-1-ol	326 (0.7) a	413 (7.9) b	358 (4.2) c	401 (5.7) b
1-hexanol	2719 (40.3) a	1485 (46.7) b	1601 (0.1) c	1138 (17.1) d
(*Z*)-3-hexen-1-ol	542 (3.5) a	523 (7.2) b	470 (0.1) c	473 (5.7) c
(*E*)-2-hexen-1-ol	6835 (183.8) a	2423 (74.3) b	3986 (14.8) c	2251 (19.6) b
		Esters		
hexyl acetate	39 (1.4) a	89 (6.6) b	32 (0.7) a	86 (1.9) b
(*Z*)-3-hexenyl acetate	165 (7.8) a	429 (11.2) b	147 (2.1) a	402 (3.8) c
		Ketones		
3-pentanone	680 (7.8) a	486 (1.3) b	471 (7.8) c	301 (0.1) d
1-penten-3-one	87 (2.8) a	384 (2) b	256 (6.4) c	357 (0.6) d

[a]The data are the mean values of two independent experiments analyzed in duplicate, and the standard deviation is reported in parentheses. The values in each row with different letters (a–d) are significantly different from one another ($p < 0.05$).

Table 8. Volatile Composition (μg/kg) of VOO-C and VOO-FTC Extracted at 30 °C for 0, 5, and 10 min of Malaxation Time Extracted from Olives of cv. *Peranzana*

compd	VOO-C	VOO-FTC (0 min)	VOO-FTC (5 min)	VOO-FTC (10 min)
		Aldehydes		
hexanal[a]	371 (12.4) a	492 (7.4) b	478 (38.4) b	594 (1.3) c
(*E*)-2-pentenal	75 (0.7) a	89 (3.5) b	90 (2.1) b	76 (1.9) a
(*E*)-2-hexenal	24038 (1412.0) a	30735 (374.9) b	32353 (152.4) b	28629 (81.3) c
		Alcohols		
1-penten-3-ol	476 (6.4) a	585 (0.9) b	600 (0.6) c	606 (2.5) c
1-pentanol	53 (3.5) a	56 (1.6) ac	36 (3.0) b	60 (0.1) c
(*E*)-2-penten-1-ol	444 (4.9) a	717 (6.4) b	679 (19.5) c	611 (14.4) d
1-hexanol	1831 (61.1) a	2703 (35.4) b	1571 (5.5) c	1853 (15.0) a
(*Z*)-3-hexen-1-ol	343 (5.6) a	446 (8.5) b	347 (9.1) a	502 (9.4) c
(*E*)-2-hexen-1-ol	1751 (64.8) a	3486 (20.2) b	3270 (6.7) c	2315 (0.6) d
		Esters		
hexyl acetate	137 (5.4) a	592 (3.8) b	482 (6.7) c	215 (8.8) d
(*Z*)-3-hexenyl acetate	443 (14.6) a	1451 (1.8) b	1234 (20.7) c	670 (13.8) d
		Ketones		
3-pentanone	580 (13.4) a	661 (24.7) b	514 (4.9) c	762 (8.8) d
1-penten-3-one	279 (1.4) a	351 (10.4) b	458 (0.1) c	331 (18.1) b

[a]The data are the mean values of two independent experiments analyzed in duplicate, and the standard deviation is reported in parentheses. The values in each row with different letters (a–d) are significantly different from one another ($p < 0.05$).

Moreover, the partial reduction of aldehydes and alcohols observed in VOO-FTC, obtained after 10 min of malaxation bolstered the hypothesis given above: comparing the head space data of VOO-FTC extracted without malaxation and those of the VOO-FTC extraction carried out for 10 min of the malaxing phase, we observed that a significant loss of (*E*)-2-hexenal (from 30.7 to 28.6 mg/kg), hexyl acetate (from 0.6 to 0.2 mg/kg), and (*Z*)-3-hexenyl acetate (from 1.5 to 0.7 mg/kg) was caused (Table 8).

The FTC treatment to the olive pastes applied on an industrial mill, with the aim of improving its working capacity, allowed a significant increase of the those volatile compounds responsible for the positive olfactory attributes[12,13] of the VOOs-FTC compared to those obtained by traditional malaxation. However, it caused a significant reduction in the percentage of the oil recovered and of the polyphenols' content as well. These negative findings were strongly reduced applying a further short malaxation period at 30 °C to the FTC pastes.

In conclusion, optimal operative conditions in terms of time and temperature applied during malaxation after the FTC treatment can be opportunely chosen for improving the relative virgin olive oil quality, according to the characteristics of olives such as the olives' cultivar, sanitary state, or maturation index or the growing climatic conditions of the drupes. Finally, FTC might have a very interesting application in those geographical zones where the olives are characterized by a very low temperature before their processing. This would cause longer malaxation time (for reaching optimal processing temperatures), which might be reduced by applying the FTC system: by rapid heating of the pastes after crushing, the gap between

the olive pulp temperature and the optimal virgin olive oil extraction process temperature would be reduced.

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ABBREVIATIONS USED

VOO, virgin olive oil; FTC, flash thermal conditioning; cryo-SEM, cryo-scanning electron microscopy; VOO-C, virgin olive oil control; VOO-FTC, virgin olive oil flash thermal conditioning; 3,4-DHPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to (3,4-dihydroxyphenyl)ethanol; *p*-HPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to (*p*-hydroxypheny1)ethanol; 3,4-DHPEA-EA, isomer of the oleuropein aglycon; 3,4-DHPEA, (3,4-dihydroxyphenyl)ethanol; *p*-HPEA, (*p*-hydroxyphenyl)ethanol; LOX, lipoxygenase; PPO, polyphenoloxidase; POD, peroxidase; PLC, programmable logic controller; HPLC, high-performance liquid chromatography; DAD, diode array detector; NMR, nuclear magnetic resonance; FH, frozen-hydrated; PVDF, polyvinylidene fluoride; FLD, fluorescence detector; HS-SPME, headspace solid-phase microextraction; GC/MS, gas chromatography−mass spectrometry; EFC, electronic flow controller; EI, electron ionization; HPL, hydroperoxide lyase

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