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Effect of Malaxation Conditions on Phenol and Volatile Profiles in Olive Paste and the Corresponding Virgin Olive Oils (*Olea europaea* L. Cv. Cornicabra)

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Malaxation of olive paste must be considered to be much more than a simple physical separation, because a complex bioprocess takes place that is very relevant to the quality and composition of the final product. A combined study of the effect of kneading temperature and time on the minor composition of olive paste and its corresponding virgin olive oil, processed in an experimental oil mill (Pieralisi, Fattoria) with a working capacity of 200 kg/h, is reported. A large drop in the oleuropein content in the olive paste with respect to its initial content in the olive fruit (between 92 and 96%) was observed, which suggested its almost total degradation during the crushing operation. The major phenolic compound found in the olive paste during kneading was the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA, always higher than 60% of the total phenols). This greatly decreased during malaxation (from 5505 to 2317 mg/kg, on average). The content of phenolic compounds in virgin olive oil was much more affected by the malaxation temperature than the kneading time. For instance, the 3,4-DHPEA-EDA content increased by 220−630% in the two batches when the temperature was increased from 20 to 40 °C. A reduction in the C6 aldehydes was found in virgin olive oil as the malaxation temperature increased, especially in *E*-2-hexenal (30% reduction). In contrast, C6 aldehydes in the oils from the oil mill plant significantly increased as the malaxation time increased from 30 to 90 min, chiefly *E*-2-hexenal (about a 70% increase).

KEYWORDS: Virgin olive oil; olive paste; phenols; volatiles; malaxation temperature; malaxation time

INTRODUCTION

The unique sensory profile of extra virgin olive oil is due to the presence of its minor components, chiefly phenolic and volatile compounds (*1*, *2*), which remain in the oil due to the use of only mechanical processes for its extraction from the fruit of the olive tree (*Olea europaea* L.). The fresh and green aroma of high-quality virgin olive oils is mainly due to the presence of volatile compounds formed by the lipoxygenase (LOX) pathway from free polyunsaturated fatty acids (*3*), whereas the phenolic components affect the taste, in particular, the positive bitter and pungent sensory attributes, and the oxidative stability, the shelf life, and the nutritional properties of the virgin olive oil (*4*). Phenolics and volatiles are therefore largely responsible for the flavor of extra virgin olive oils and to a large extent determine the degree of consumer preference for this highly appreciated product.

The phenolic and volatile contents and profiles of fresh virgin olive oils depend on both (1) the initial composition of the olive fruits used for processing and (2) the technological variables used during the oil mill process, in particular, milling and malaxation conditions. Several studies have pointed out the importance of the different virgin olive oil processing stages for the minor composition found in the final product. Thus, the use of mechanical crushers leads to a higher phenol extraction from the vegetable tissues than the traditional stone mills (*5*), and therefore oils with higher antioxidant capacity are obtained (*6*). Servili et al. (*7*) also reported changes in the volatile composition and pigment content of the oil depending on the type of mill employed.

It is also important to note the great effect of the malaxation process on the olive paste; indeed, kneading must be considered to be much more than a simple physical separation, because a complex bioprocess takes place that is very relevant to the quality and composition of the final product. Indeed, malaxation conditions affect not only the oil yield but in particular the composition and quality of the final virgin olive oil. For example, it is known that kneading conditions may modify the phenol (*6*, *8*, *9*) and volatile (*8*, *10*, *13*) contents in virgin olive oil and as a consequence its properties. However, there have been only a few investigations (*14*) that have considered changes in both phenol and volatile components in response to malaxation temperature and time conditions.

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The aim of this study was therefore to improve the scientific and technological knowledge on the variables involved in the kneading operation that affect the minor components of the Cornicabra virgin olive oil. The ultimate goal was to define the optimum kneading conditions in the Cornicabra variety grown in Castilla-La Mancha for use in the industrial oil mills of our region. Such optimum temperature and time conditions during malaxation should be applied to enhance the sensory characteristics, in particular, and therefore the consumers' preference for this product.

MATERIALS AND METHODS

Experimental Oil Mill Plant and Sampling. The technological assays were performed using an oil mill plant (Pieralisi, Fattoria) with a working capacity of 200 kg/h olive paste and equipped with an olive washing machine, a hammer crusher, a single-stage kneader, a two-phase horizontal decanter, and a vertical centrifuge.

Two batches of cv. Cornicabra fruit, each weighing approximately 1600 kg provided by the same producer (Cooperativa Montes Norte, SL, Malagón, Ciudad Real) on different days, were used. The malaxation assays were performed at different temperatures (20, 28, and 40 °C) and medium residence time (30, 60, and 90 min); batches of 200 kg of fruit were processed in each technological assay. To study the evolution of phenolic and volatile compounds throughout the kneading process, three samples of olive paste were withdrawn at a residence time in the malaxer corresponding to the 25th, 50th, and 75th percentiles of the total loading−mixing−downloading time for each assay, for instance, at 47 min (P_{25}), 60 min (P_{50}), and 73 min (P_{75}) for a medium residence time of 60 min. Olive pomace samples were also taken to analyze the phenolic composition in the tissue residues after centrifugation of the olive paste; all samples were frozen and stored at −70 °C until analysis. All virgin olive oil samples produced in each assay were filtered with anhydrous Na_2SO_4 and stored at 10 °C in darkness using amber glass bottles without headspace prior to analysis. In addition, separate assays using the same olive batches were carried out using the laboratory scale Abencor system, with the aim of comparing the results obtained with the oil mill plant and the laboratory scale extraction system and to widen the temperature−time conditions studied (20, 24, 28, 35, and 40 °C and 15, 30, 45, 60, and 75 min).

Analytical Determinations in Olive Fruits, Olive Paste, and Olive Pomace. The water content of olive fruit was determined by desiccation according to UNE Spanish Standard method 55032:1973 (*15*). The fat content was determined by Soxhlet extraction and was expressed as a percentage of dry olive paste weight (*15*).

The phenolic composition of olive fruit, olive paste, or olive pomace was obtained through the following procedure: 4.0 g of sample was homogenized with a mixture of methanol/water (80:20 v/v) (40 mL) for 2 min with an Ultraturrax homogenizer (14000 rpm). The suspension obtained was shaken (20 min, 150 rpm, < 4 °C in darkness) and then centrifuged (10 min at 4000*g* and 4 °C). The hydromethanolic phase was recovered and filtered with a 0.45 μm nylon syringe filter.

The phenolic fraction extracted was analyzed by high-performance liquid chromatography (HPLC) using an Agilent Technologies 1100 series system equipped with an automatic injector, a column oven, and a diode array UV detector. A Zorbax SB-C18 column (250 × 4.6 id mm, 5 μm particle size) (Agilent Technologies, Palo Alto, CA), maintained at 30 °C, was used with an injection volume of 20 μL and a flow rate of 1.0 mL/min. The mobile phase was a mixture of water/acetic acid (95:5 v/v) (solvent A), methanol (B), and acetonitrile (C) from 95% (A)−2.5% (B)−2.5% (C) to 34% (A)−33% (B)−33% (C) in 50 min. Chromatograms were recorded at 280, 340, and 520 nm. The hydroxytyrosol, oleuropein, and demethyloleuropein from olive fruit and secoiridoids of hydroxytyrosol and tyrosol from the olive paste and pomace were quantified at 280 nm, the anthocyanins at 520 nm, and the verbascoside and flavonoids at 340 nm. Phenolic compound quantification was achieved by a minimum of a five-point calibration curve on the basis of the corresponding standard substances or on the basis of their response factors (*17*), with the exception of demethyloleuropein and anthocyanins from olive fruit, which were quantified as oleuropein.

The identification of noncolored phenolic compounds was carried out by comparison of their retention times, UV−visible characteristics, and MS spectra with their standard substances. In the case of the anthocyanins and demethyloleuropein, these were tentatively identified by their UV−visible characteristics and MS spectra. The mass detector used was an LCQ Deca XP Plus (Thermo Electron Corp., Waltham, MA) equipped with an electrospray ionization system. Nitrogen was used as a nebulizing gas at a flow rate of 14 units. The temperature and voltage of the capillary were 250 °C and 4.50 kV, respectively. Data were acquired in the negative ionization mode. Fragmentation experiments were performed using helium as the collision gas with collision energy between 30 and 40%.

Volatile Compounds [Adapted from Vichi et al. (*16*)]. Solid-phase microextraction (SPME) followed by GC-FID was used to analyze the volatile compounds in the olive paste samples studied. One and a half grams of olive paste was placed in a 10 mL vial fitted with a silicone septum. SPME sampling was performed by exposing the DVB/Carboxen/PDMS fiber (50/30 μm, 2 cm long from Supelco Inc., Bellefonte, PA) for 30 min in the headspace of the sample maintained at 40 °C and then retracted into the needle and immediately transferred and desorbed for 5 min in the injection port of a gas chromatograph equipped with an FID. Compounds were resolved on a Supelcowax-10 column (30 m × 0.25 mm × 0.25 μm) under the following conditions: injection port temperature, 260 °C; helium flow, 0.8 mL/min; oven temperature ramp, 35 °C for 10 min, 3 °C/min to 160 °C, and then 15 °C/min to 200 °C (maintained for 5 min). Volatile compounds were identified by comparison of retention times and mass spectra of standard substances (Sigma Aldrich) added to refined olive oil. The equipment used was an Agilent 5975C series mass spectrometer (Agilent Technologies) equipped with an electron ionization (EI+) detector and coupled to an Agilent 6850 series gas chromatograph. The capillary column used was a DB-Wax (30 m × 0.25 mm × 0.25 μm, J&W Scientific, Folsom, CA). Helium was employed as the carrier gas at a flow rate of 0.8 mL/min. The transfer line temperature was 280 °C, and the temperatures of the ionization source and the quadrupole were 230 and 150 °C, respectively, with an electromultiplier voltage of +941 eV. Their quantification was achieved by a minimum of a five-point calibration curve on the basis of the corresponding standard substances.

Analytical Determinations in Virgin Olive Oil. All reagents used were of analytical, HPLC, or spectroscopic grade and were supplied by Merck (Darmstadt, Germany).

Phenolic Compounds. A solution of the internal standard (250 μL of 15 mg/kg of syringic acid in methanol) was added to a sample of virgin olive oil (2.5 g), and the solvent was evaporated with a rotary evaporator at 35 °C under vacuum. The oil was then dissolved in 6 mL of hexane, and a diol-bonded phase cartridge (Supelco Co.) was used to extract the phenolic fraction. The cartridge was conditioned with methanol (6 mL) and hexane (6 mL), the oil solution was then applied, and the SPE column was washed with hexane (2 × 3 mL) and with hexane/ethyl acetate (85:15, v/v; 4 mL). Finally, the phenols were eluted with methanol (15 mL), and the solvent was removed with a rotary evaporator at 35 °C under vacuum until dryness. The phenolic residue was dissolved in methanol/water (1:1 v/v; 250 μL) and analyzed by HPLC. Chromatographic conditions were the same as those used with the olive pulp and olive pomace phenolic fractions. Phenolic compounds were quantified at 280 nm using syringic acid as the internal standard and the response factors determined by Mateos et al. (*17*).

Oleocanthal was extracted by liquid−liquid extraction following the method developed by Impellizzeri et al. (*18*). A solution of the internal standard (250 μL of 200 mg/kg of 3,5-dimethoxyphenol in methanol) was added to a sample of virgin olive oil (1.0 g), and the solvent was evaporated with a rotary evaporator at 35 °C under vacuum. The oil was then dissolved in 2 mL of hexane and transferred to a centrifuge tube (15 mL). The tube was shaken for 15 s twice, then 5 mL of acetonitrile was added, and shaking was repeated twice for 15 s. The tube was centrifuged at 4000 rpm for 5 min to separate the solvent from the oil phase, and the solvent extract was collected in another centrifuge tube. Each sample was extracted three times in this way, and the solvent of the combined extract was removed with a N_2 stream. The residue of the extract was dissolved with 1 mL of methanol/water (1:1, v/v). Hexane (1 mL) was added to the solution to wash away any remaining oil; this procedure was carried out

three times. The tube was centrifuged, and the methanolic phase was recovered.

The fraction extracted was analyzed by HPLC using an Agilent Technologies 1100 series system equipped with an automatic injector, a column oven, and a diode array UV detector. A Zorbax SB-C18 column (250 × 4.6 id mm, 5 μm particle size) (Agilent Technologies), maintained at 25 °C, was used with an injection volume of 20 μL and a flow rate of 1.0 mL/min. The mobile phase was a mixture of acetonitrile (A) and water (B) from 25% (A)−75% (B) for 35 min to 80% (A)−20% (B) in 0.01 min for 10 min and returned to the initial conditions in 0.01 min. Oleocanthal was quantified at 280 nm using 3,5-dimethoxyphenol as the internal standard.

Volatile Compounds. SPME followed by GC-FID was used to analyze the volatile compounds in the virgin olive oil samples studied. One and a half grams of olive oil was placed in a 10 mL vial fitted with a silicone septum. The SPME sampling and CG-FID conditions, as well as identification and quantification of volatiles, were the same as those used with the olive paste volatile fraction.

All experiments and analytical determinations were carried out in duplicate.

Statistical Analysis. Analysis of ANOVA was performed using SPSS 15 statistical software (SPSS Inc., Chicago, IL). Duncan's test ($p < 0.05$) was used to discriminate among the mean values.

RESULTS AND DISCUSSION

The influence of temperature and time conditions on (1) oil yield and (2) overall quality indices (e.g., free acid content, peroxide index, K_{232}, K_{270}) on Cornicabra virgin olive oil has been analyzed and discussed elsewhere (*19*).

Evolution of Biophenols in the Olive Paste during Malaxation. The initial characteristics of the two batches of Cornicabra olive fruit employed in this assay are reported in **Table 1**, which shows a similar composition with respect to oil yield, humidity, dry matter, and biophenol content in the batches used in this study, with the exception of the oleuropein concentration, which was about 18% higher in batch I. As previously reported, this oleoside is by far the most abundant biophenol in the olive fruit (14043 and 11924 mg/kg in batches I and II, respectively) (*19*−*22*) and the main precursor of the phenolic compounds formed during crushing and malaxation and present in virgin olive oil (*23*).

Table 1 also reports the phenolic composition of the olive paste obtained a few minutes (3−4 min, half malaxer loading time) after the crushing stage. It shows a large drop in the oleuropein content in the olive paste with respect to its original content in the olive fruit (between 92 and 96%), which suggested its almost total degradation and transformation during the crushing operation. This was probably caused by the activity of the β-glucosidase enzyme, confirmed by the presence of relevant quantities of the different secoiridoid derivatives in the olive paste, especially the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) with concentrations of 6979 and 4782 mg/kg, respectively, in the two batches studied.

The content of the individual phenols in the olive paste under the different kneading conditions studied in the oil mill plant are shown in **Tables 2** and **3**. As expected, the phenolic compounds found in the olive paste were a mixture of the biophenols found in the olive fruit and in the final virgin olive oil (VOO).

With regard to the content of secoiridoid compounds, there was a noticeable decrease in oleuropein with time during the malaxation process (from 790 to 270 mg/kg, on average, for batches I and II; **Figure 1**), probably due to the β-glucosidase activity during the kneading of olive paste, as also reported by Artajo et al. (*24*).

Table 1. Olive Fruit and Olive Paste Initial Composition of the Two Cv. Cornicabra Batches Studied

	fruit	paste $t = 0$[a]	fruit	paste $t = 0$
	Batch I		Batch II	
ripeness index	4.5 ± 0.3		4.7 ± 0.2	
oil yield (%, FW)	24.6 ± 0.4		28.4 ± 0.3	
humidity (%)	39.8 ± 0.8		37.5 ± 0.7	
phenolic compounds (mg/kg)				
oleuropein	14042 ± 209	1071 ± 32	11924 ± 639	508 ± 17
demethyloleuropein	nd[b]	nd	nd	nd
hydroxytyrosol	202 ± 45	155 ± 1	204 ± 22	223 ± 2
3,4-DHPEA-EDA	nd	6979 ± 21	nd	4782 ± 41
3,4-DHPEA-EA	nd	448 ± 7	nd	430 ± 8
tyrosol	nd	tr[c]	nd	tr
p-HPEA-EDA	nd	467 ± 9	nd	553 ± 15
p-HPEA-EA	nd	89 ± 4	nd	56 ± 2
verbascoside	131 ± 4	116 ± 3	63 ± 6	54 ± 4
rutin	343 ± 7	166 ± 2	331 ± 11	116 ± 3
luteolin-7-*O*-glucoside	319 ± 85	119 ± 5	351 ± 48	103 ± 3
apigenin-7-*O*-glucoside	68 ± 11	49 ± 3	62 ± 9	61 ± 1

[a] $t = 0$, olive paste obtained a few minutes after crushing (half malaxer loading time). [b] nd, not detected. [c] tr, traces.

The major phenolic compound found in the olive paste during the kneading phase was the hydroxytyrosol derivative 3,4-DHPEA-EDA, which always accounted for >60% of the total phenols present in the olive paste (**Tables 2** and **3**). The concentration of this compound greatly decreased during malaxation (from 5505 to 2317 mg/kg, on average, for batches I and II, respectively), probably due to the activity of oxidative enzymes such as polyphenol oxidase (PPO), peroxidase (POD), or lipoxygenase (LOX) (*25*). As a consequence, the secoiridoid derivatives of hydroxytyrosol are the olive paste biophenols that are most affected by time during malaxation, as clearly depicted in **Figure 1**.

In contrast, the other secoiridoid derivatives found in the olive paste were present at much lower average levels (7.6% of the total phenols for 3,4-DHPEA-EA, 11.6% for *p*-HPEA-EDA, and 0.8% for *p*-HPEA-EA) and furthermore did not show any clear trend with time during kneading (**Table 2**), with the exception of a slight decrease in 3,4-DHPEA-EA.

It is important to note the known hydrophilic character of the simple phenols and their secoiridoid derivatives, as clearly observed by the higher content of these compounds found in the wet pomace compared to the olive oil (**Figure 2**). For example, the hydroxytyrosol (3,4-DHPEA) found in the kneaded olive paste was almost totally retained in the pomace after centrifugation (99.8% of its total content), whereas the contents of 3,4-DHPEA-EDA, 3,4-DHPEA-EA, *p*-HPEA-EDA, and *p*-HPEA-EA in the olive oil compared to their absolute content in the kneaded olive paste were 1, 10, 7, and 53%, respectively. Furthermore, tyrosol (*p*-HPEA) is present in significant amounts only in virgin olive oil, with only traces being detected in the kneaded paste and pomace.

The concentrations of verbascoside and flavonoids, such as rutin, luteolin-7-*O*-glucoside, and apigenin-7-*O*-glucoside, barely changed with time during the malaxation process (**Table 2**) and remained the same in the pomace (**Table 2** and **Figure 2**), as also described by Artajo et al. (*24*).

The effect of malaxation temperature on the phenolic composition of the olive paste is reported in **Table 3**. The content of oleuropein and secoiridoid derivatives of hydroxytyrosol (3,4-DHPEA-EDA and 3,4-DHPEA-EA) significantly

Table 2. Effect of Malaxation Time on Biophenols (Milligrams per Kilogram) in the Olive Paste (Kneading Temperature = 28 °C)[a]

		30 min			60 min			90 min		
		17 min (P_{25})	30 min (P_{50})	43 min (P_{75})	47 min (P_{25})	60 min (P_{50})	73 min (P_{75})	77 min (P_{25})	90 min (P_{50})	103 min (P_{75})
phenolic alcohol										
3,4-DHPEA	I	165 ± 1b,w	163 ± 2a,w	176 ± 2a,x	181 ± 1c,w	189 ± 3b,x	201 ± 3c,y	156 ± 2a,w	186 ± 3b,x	181 ± 2b,x
	II	211 ± 2b,w	210 ± 2c,w	234 ± 2a,x	146 ± 1a,w	186 ± 3b,x	237 ± 3a,y	224 ± 2c,x	175 ± 3a,w	258 ± 3b,y
p-HPEA	I	tr[b]	tr	tr	tr	tr	tr	tr	tr	tr
	II	tr	tr	tr	tr	tr	tr	tr	tr	tr
secoiridoids										
oleuropein	I	1187 ± 5b,y	714 ± 8c,x	471 ± 9c,w	418 ± 4a,x	383 ± 7a,w	371 ± 6b,w	406 ± 9a,x	407 ± 3b,x	318 ± 2a,w
	II	392 ± 7b,y	335 ± 7c,x	260 ± 7b,w	312 ± 8a,w	293 ± 8b,w	288 ± 9b,w	299 ± 2a,y	245 ± 3a,x	227 ± 3a,w
ligstroside	I	nd[c]	nd	nd	nd	nd	nd	nd	nd	nd
	II	nd	nd	nd	nd	nd	nd	nd	nd	nd
secoiridoid derivatives										
3,4-DHPEA-EDA	I	6355 ± 6c,y	6055 ± 9c,x	4941 ± 8c,w	4798 ± 14b,y	4113 ± 10a,x	3647 ± 5b,w	4334 ± 8a,y	4249 ± 9b,x	3023 ± 8a,w
	II	4655 ± 8c,y	3678 ± 8c,x	3242 ± 9c,w	2762 ± 5b,y	2677 ± 7b,x	2584 ± 7b,w	2616 ± 5a,y	2077 ± 9a,x	1611 ± 5a,w
3,4-DHPEA-EA	I	458 ± 5c,w	458 ± 3c,w	473 ± 4c,x	441 ± 2b,x	436 ± 2b,x	418 ± 4b,w	415 ± 4a,x	423 ± 2a,x	333 ± 4a,w
	II	415 ± 3b,x	444 ± 7c,y	400 ± 2c,w	322 ± 5a,w	360 ± 1b,x	376 ± 3b,y	431 ± 2c,y	292 ± 2a,w	340 ± 5a,x
p-HPEA-EDA	I	518 ± 5c,w	539 ± 3c,w	618 ± 4c,x	635 ± 2b,x	631 ± 2b,x	644 ± 4b,w	738 ± 7c,x	715 ± 9c,x	704 ± 9c,w
	II	542 ± 6b,w	560 ± 7a,x	571 ± 2a,y	514 ± 8a,w	572 ± 7a,x	569 ± 9a,x	584 ± 9b,w	634 ± 4b,x	638 ± 9b,x
p-HPEA-EA	I	84 ± 1b,y	62 ± 2c,x	57 ± 1c,w	53 ± 2a,x	51 ± 3a,w	51 ± 1b,w	50 ± 1a,w	56 ± 1b,x	47 ± 2a,w
	II	50 ± 1b,w	48 ± 1c,w	51 ± 1c,w	33 ± 2a,w	36 ± 2b,w	41 ± 2b,x	31 ± 1a,w	27 ± 2a,w	37 ± 2a,x
verbascoside	I	115 ± 3a,w	119 ± 2a,w	121 ± 2a,w	112 ± 3a,w	118 ± 7a,w	123 ± 5a,x	113 ± 2a,w	115 ± 3a,w	119 ± 2a,w
	II	55 ± 2a,w	56 ± 1a,w	59 ± 2a,w	56 ± 2a,w	58 ± 4a,w	56 ± 3a,w	56 ± 1a,w	55 ± 2a,w	58 ± 1a,w
flavonoids										
rutin	I	150 ± 2b,y	141 ± 2b,x	127 ± 2c,w	112 ± 2a,w	111 ± 3a,w	109 ± 2b,w	108 ± 2a,x	109 ± 2a,x	101 ± 2a,w
	II	111 ± 2b,x	120 ± 2c,y	106 ± 2a,w	105 ± 3a,w	108 ± 2b,w	106 ± 3a,w	108 ± 2a,x	103 ± 2a,w	108 ± 2a,x
luteolin-7-*O*-glucoside	I	115 ± 2b,w	118 ± 2b,x	118 ± 1a,x	108 ± 2a,w	111 ± 3a,w	116 ± 3a,x	112 ± 2b,w	115 ± 2b,x	116 ± 2a,x
	II	94 ± 3a,w	111 ± 1b,y	105 ± 2b,x	102 ± 2b,x	100 ± 2a,w	98 ± 1a,w	101 ± 1b,w	100 ± 1a,w	108 ± 2b,x
apigenin-7-*O*-glucoside	I	50 ± 3a,w	52 ± 2a,w	57 ± 1a,x	55 ± 4a,w	56 ± 3a,w	57 ± 3a,w	53 ± 2a,w	55 ± 2a,w	56 ± 1a,w
	II	59 ± 1a,x	57 ± 3a,x	51 ± 2a,w	60 ± 2a,x	58 ± 2a,x	50 ± 3a,w	55 ± 3a,w	54 ± 2a,w	55 ± 2a,w

[a] Different letters within a column (a−c) indicate significant differences ($p < 0.05$) with respect to malaxation time in olive paste. Different letters within a column (w−y) indicate significant differences ($p < 0.05$) with respect to olive paste at different stages of malaxation process. [b] tr, traces. [c] nd, not detected.

decreased with temperature [from 401 to 316 mg/kg, from 3862 to 3635 mg/kg, and from 451 to 406 mg/kg, respectively, from 20 to 40 °C at the same residence malaxation time of 60 min (P_{50}); **Table 3**], whereas *p*-HPEA-EDA content showed the opposite trend (from 529 to 773 mg/kg under the same kneading conditions). These data apparently confirm the considerable oxidative degradation, both chemical and enzymatic, of the compounds with an ortho-diphenolic structure during malaxation in the presence of oxygen due to LOX activity (*11*) and PPO activity, which possesses a higher affinity for the *o*-diphenols than monophenols (*26*). Verbascoside and flavonoids were not visibly affected by the processing temperature.

Effect on Virgin Olive Oil Phenols. Figures 3 and **4** depict the effect of kneading temperature and time on hydroxytyrosol, tyrosol, and their secoiridoid derivatives in the VOO obtained in the experimental oil mill plant and in the laboratory.

There was a significant increase in the secoiridoids of hydroxytyrosol and tyrosol as the malaxation temperature rose from 20 to 40 °C. In particular, the 3,4-DHPEA-EDA content increased between 220 and 630% in each of the two batches studied in the oil mill plant (**Figure 3a**; individual data not shown). This observed trend was opposite that found in the olive paste during kneading, probably due to an increase in the partition coefficient of the phenolic compounds between the oily and water phases of the olive paste (*27*). Other investigations using industrial olive oil mills found a similar trend (*8*, *28*).

Similar behavior in the VOO phenol content was observed in samples obtained under different malaxation temperature conditions using the laboratory scale Abencor system (**Figure 4a**), with an increase of 300 and 110%, on average, between batches I and II, for 3,4-DHPEA-EDA and *p*-HPEA-EDA. In contrast, other studies carried out in laboratory setups (batches lower than 10 or 20 kg) reported a decrease in the phenol content of the oil as the kneading temperature of the olive paste increased (*10*, *29*). According to these authors, despite the reported rise in the partition coefficient of phenolic compounds, the reduction in these compounds is due to oxidation, because the small quantity of olive paste kneaded promoted excessive contact with atmospheric oxygen, although this was not observed in the current work. It is very interesting that using a higher number of experimental temperatures, as carried out in this study at the laboratory scale, demonstrated an important increase in the olive oil phenolic content within the narrow interval of 24−28 °C (**Figure 4a**).

On the other hand, as the malaxation time increased, there was a small decrease in the secoiridoid derivatives of hydroxytyrosol of about 5%, whereas the content of secoiridoids of tyrosol increased by between 15 and 20% in the oil mill plant (**Figure 3b**). These trends were similar to those observed in the olive paste during malaxation. Similar results were found by Di Giovacchino et al. (*6*), Angerosa et al. (*10*), and Lercker et al. (*12*). Moreover, these tendencies were also observed in the oil samples processed in the Abencor system, although the decrease in the derivatives of hydroxytyrosol and the increase in those from tyrosol were somewhat higher, at 45 and 50%, respectively, on average, for batches I and II (**Figure 4b**).

It is therefore apparent that the content of phenolic compounds in virgin olive oil is much more affected by the malaxation temperature than the kneading time (**Figures 3** and **4**).

It is well-known that the phenolic composition affects the organoleptic properties of VOO. For example, the intensity of the sensory perception of pungency is mainly related to the

Table 3. Effect of Malaxation Temperature on the Biophenols (Milligrams per Kilogram) in the Olive Paste (Median Kneading Time = 60 min)[a]

		20 °C			28 °C			40 °C		
		47 min (P_{25})	60 min (P_{50})	73 min (P_{75})	47 min (P_{25})	60 min (P_{50})	73 min (P_{75})	47 min (P_{25})	60 min (P_{50})	73 min (P_{75})
phenolic alcohols										
3,4-DHPEA	I	212 ± 1c,w	233 ± 2c,x	248 ± 3c,y	181 ± 1b,w	189 ± 3b,x	201 ± 3b,y	159 ± 2a,w	172 ± 8a,x	182 ± 3a,y
	II	225 ± 2c,w	241 ± 2c,x	242 ± 2c,x	146 ± 1a,w	186 ± 3a,x	237 ± 3b,y	196 ± 3b,x	197 ± 1b,x	172 ± 2a,w
p-HPEA	I	tr[b]	tr	tr	tr	tr	tr	tr	tr	tr
	II	tr	tr	tr	tr	tr	tr	tr	tr	tr
secoiridoids										
oleuropein	I	584 ± 9c,y	408 ± 9c,x	372 ± 9b,w	418 ± 4a,x	383 ± 7b,w	371 ± 6b,w	468 ± 9b,x	316 ± 5a,w	323 ± 2a,w
	II	421 ± 8c,y	393 ± 6c,x	375 ± 7b,w	312 ± 8a,w	293 ± 8a,w	288 ± 9a,w	386 ± 4b,x	315 ± 7b,w	307 ± 3b,w
ligstroside	I	nd[c]	nd	nd	nd	nd	nd	nd	nd	nd
	II	nd	nd	nd	nd	nd	nd	nd	nd	nd
secoiridoid derivatives										
3,4-DHPEA-EDA	I	4318 ± 8b,y	4012 ± 7b,x	3547 ± 6b,w	4798 ± 14b,y	4113 ± 10a,x	3647 ± 5b,w	3431 ± 9a,y	3296 ± 7a,w	3346 ± 8a,x
	II	4177 ± 8b,y	3712 ± 8c,x	3059 ± 6c,w	2762 ± 5b,y	2677 ± 7b,x	2584 ± 7b,w	2898 ± 6a,x	2974 ± 6b,y	2363 ± 9a,w
3,4-DHPEA-EA	I	482 ± 4b,w	495 ± 3b,x	479 ± 5c,w	441 ± 2b,x	436 ± 2b,x	418 ± 4b,w	451 ± 2c,y	440 ± 5a,w	464 ± 3b,x
	II	441 ± 5c,y	408 ± 6c,x	402 ± 5c,w	322 ± 5a,w	360 ± 1b,x	376 ± 3b,y	390 ± 5b,y	373 ± 4b,x	344 ± 3a,w
p-HPEA-EDA	I	473 ± 3a,w	563 ± 3a,x	562 ± 9a,x	635 ± 2b,x	631 ± 2b,x	644 ± 4b,w	628 ± 3b,w	736 ± 4c,x	787 ± 5c,y
	II	495 ± 6a,x	494 ± 2a,x	485 ± 5a,w	514 ± 8a,w	572 ± 7a,x	569 ± 9a,x	766 ± 7b,w	810 ± 9c,x	771 ± 9c,w
p-HPEA-EA	I	35 ± 1b,w	52 ± 1b,x	47 ± 1a,x	53 ± 2a,x	51 ± 3a,w	51 ± 1b,w	20 ± 2a,w	30 ± 2a,x	57 ± 1b,y
	II	56 ± 1b,x	49 ± 1b,x	38 ± 1a,w	33 ± 2a,w	36 ± 2b,w	41 ± 2b,x	62 ± 2c,x	57 ± 2c,x	37 ± 1a,w
verbascoside	I	123 ± 3b,w	126 ± 2b,w	125 ± 3a,w	112 ± 3a,w	118 ± 7a,w	123 ± 5a,x	115 ± 3a,w	121 ± 2a,x	127 ± 3a,x
	II	55 ± 2a,w	57 ± 3a,w	58 ± 2a,w	56 ± 2a,w	58 ± 4a,w	56 ± 3a,w	55 ± 4a,w	57 ± 1a,w	58 ± 2a,w
flavonoids										
rutin	I	141 ± 2c,w	139 ± 2c,w	139 ± 2c,w	112 ± 2b,w	111 ± 3b,w	109 ± 2b,w	102 ± 2a,w	99 ± 1a,w	97 ± 1a,w
	II	135 ± 1b,x	138 ± 2c,x	126 ± 2c,w	105 ± 3a,w	108 ± 2b,w	106 ± 3b,w	108 ± 2a,x	100 ± 1a,w	94 ± 2a,w
luteolin-7-*O*-glucoside	I	97 ± 3a,w	101 ± 1a,w	108 ± 2a,x	108 ± 2b,w	111 ± 3b,w	116 ± 3b,x	107 ± 3b,w	107 ± 2b,w	111 ± 2a,w
	II	89 ± 1a,w	95 ± 2a,x	96 ± 1a,x	102 ± 2b,x	100 ± 2b,w	98 ± 1a,w	116 ± 9c,w	111 ± 2c,w	114 ± 3b,w
apigenin-7-*O*-glucoside	I	56 ± 1a,w	58 ± 2a,w	54 ± 2a,w	55 ± 4a,w	56 ± 3a,w	57 ± 3a,w	57 ± 2a,w	53 ± 1a,w	50 ± 4a,w
	II	55 ± 2a,w	57 ± 1a,w	60 ± 1a,w	60 ± 2a,x	58 ± 2a,x	50 ± 3a,w	59 ± 2a,w	57 ± 1a,w	58 ± 2a,w

[a] Different letters within a column (a−c) indicate significant differences ($p < 0.05$) with respect to malaxation temperature in olive paste. Different letters within a column (w−y) indicate significant differences ($p < 0.05$) with respect to olive paste at different stages of malaxation process. [b] tr, traces. [c] nd, not detected.

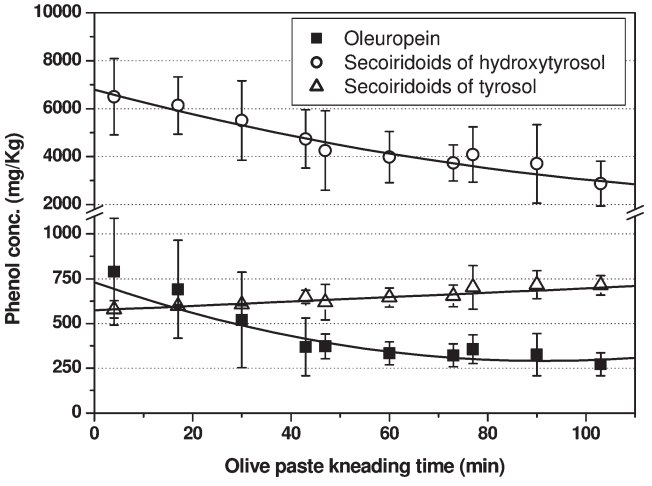

Figure 1. Evolution of oleuropein and hydroxytyrosol and tyrosol secoiridoids in the olive paste with malaxation time at 28 °C: ■, oleuropein; ○, secoiridoids of hydroxytyrosol; △, secoiridoids of tyrosol.

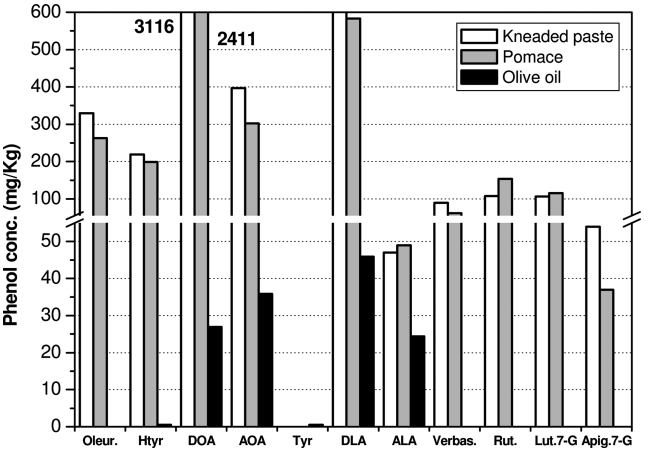

Figure 2. Distribution of phenolic compounds (mg/kg) in kneaded paste, pomace, and virgin olive oil. Oleur., oleuropein; Htyr, hydroxytyrosol; DOA, 3,4-DHPEA-EDA; AOA, 3,4-DHPEA-EA; Tyr, tyrosol; DLA, *p*-HPEA-EDA; ALA, *p*-HPEA-EA; Verbas., verbascoside; Rut., rutine; Lut. 7-G, luteolin-7-*O*-glucoside; Apig. 7-G, apigenin-7-*O*-glucoside.

content of the deacetoxy form of *p*-HPEA-EDA (*30*); moreover, the ibuprofen-like activity of this compound, also called oleocanthal, has recently been discovered (*31*). It was therefore of particular interest to study the evolution of this bioactive compound in VOO in response to kneading conditions. Up to 3 times higher levels were found in VOO processed in the oil mill plant after the malaxation temperature was increased from 20 to 40 °C or the kneading time was increased from 30 to 90 min (**Figure 5a**), and similar trends were also observed in the oils obtained in the laboratory (**Figure 5b**). On the other hand, the secoiridoid derivatives of hydroxytyrosol, especially 3,4-DHPEA-EDA and 3,4-DHPEA-EA, are associated with the sensory intensity of the bitterness attribute in the VOO (*32*), in addition to their important role as antioxidants, and, consequently, high levels of these compounds may produce excessive bitterness that may reduce the consumers' preference for the product.

Therefore, the trends observed in the phenolic composition of the virgin olive oils under the different malaxation conditions

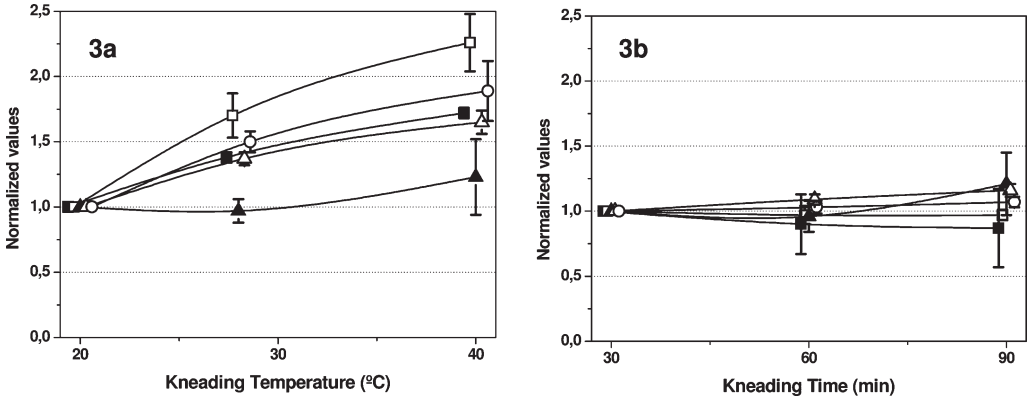

Figure 3. Effect of malaxation temperature (**a**) and time (**b**) on oil mill plant VOO phenolic profile: ■, hydroxytyrosol; □, secoiridoids of hydroxytyrosol; ▲, tyrosol; △, secoiridoids of tyrosol; ○, total phenols. Normalized data are plotted with respect to those obtained at 20 °C/60 min (**a**) and at 30 min/28 °C (**b**), on average, for the two batches.

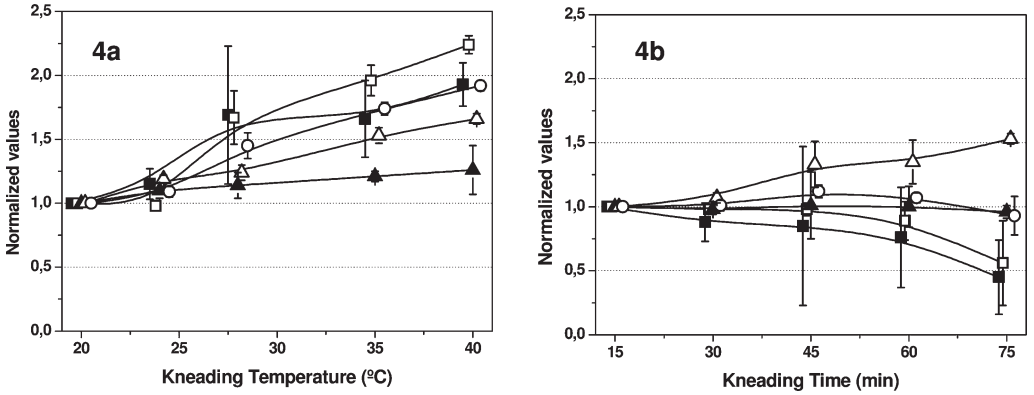

Figure 4. Effect of malaxation temperature (**a**) and time (**b**) on Abencor VOO phenolic profile: ■, hydroxytyrosol; □, secoiridoids of hydroxytyrosol; ▲, tyrosol; △, secoiridoids of tyrosol; ○, total phenols. Normalized data are plotted with respect to those obtained at 20 °C/60 min (**a**) and at 30 min/28 °C (**b**), on average, for the two batches.

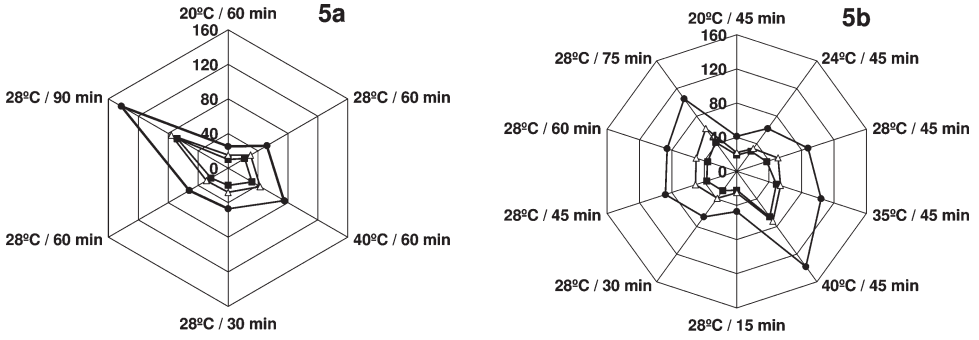

Figure 5. Effect of malaxation temperature and time on oleocanthal content (mg/kg of IS) in VOO produced in the oil mill plant (**a**) and in the Abencor system (**b**): ■, *cis*-oleocanthal; △, *trans*-oleocanthal; ●, total oleocanthal (*cis* + *trans*).

employed may be very relevant for the olive oil industry, because the control of these technological variables can provide a desirable level of these components to improve nutritional value or modify the intensity of the sensory attributes.

Evolution of Volatile Compounds in Olive Paste during Malaxation. The content of the six-carbon (C6) family of volatile compounds in the olive paste under the different malaxation temperature−time conditions studied in the oil mill plant is reported in **Tables 4** and **5**. These compounds, which are responsible for the positive green sensory notes of the virgin olive oil aroma, are produced through the LOX pathway from linoleic and linolenic acids, mainly during the crushing of the olive fruit, and are then incorporated into the oily phase during the malaxation of the olive paste (*3*).

A decrease in the C6 aldehydes in the olive paste, mainly of hexanal [from 0.27 to 0.08 mg/kg, on average, between malaxation time 0 and 17 min (P_{25})], which is related to the apple and green fruity attributes, and *E*-2-hexenal (from 1.50 to 1.07 mg/kg), which is responsible for the almond and green sensory notes, was observed just after crushing of the olive fruits and the initial kneading stage (**Table 4**). This is due to their transformation into the corresponding C6 alcohols, hexan-1-ol, *Z*-3-hexen-1-ol, and *E*-2-hexen-1-ol (related to fruity, green, grassy, and sweet notes). Nevertheless, the content of these volatiles did not further decrease in the olive paste during the kneading process (malaxation times P_{25}, P_{50}, and P_{75}), which could indicate the higher activity of the LOX pathway enzymes only at the beginning of this operation. C6 esters, such as hexyl

Table 4. Effect of Malaxation Time on LOX Volatiles (Milligrams per Kilogram) in the Olive Paste (Kneading Temperature = 28 °C)[a]

		30 min				60 min			90 min		
		time = 0	17 min (P_{25})	30 min (P_{50})	43 min (P_{75})	47 min (P_{25})	60 min (P_{50})	73 min (P_{75})	77 min (P_{25})	90 min (P_{50})	103 min (P_{75})
hexanal	I	0.32 ± 0.02	0.05 ± 0.01a,w	0.05 ± 0.01a,w	0.11 ± 0.01a,x	0.06 ± 0.01a,w	0.04 ± 0.02a,w	0.10 ± 0.01a,x	0.09 ± 0.01b,w	0.08 ± 0.01b,w	0.13 ± 0.01b,w
	II	0.22 ± 0.01	0.11 ± 0.01a,w	0.13 ± 0.01b,x	0.14 ± 0.01a,x	0.14 ± 0.01b,w	0.12 ± 0.01a,w	0.14 ± 0.01b,w	0.12 ± 0.01a,w	0.11 ± 0.01a,w	0.15 ± 0.01a,x
hexan-1-ol	I	0.35 ± 0.01	0.49 ± 0.02b,w	0.48 ± 0.01b,w	0.48 ± 0.01b,w	0.46 ± 0.01b,w	0.47 ± 0.02b,w	0.46 ± 0.01a,w	0.44 ± 0.02a,w	0.44 ± 0.01a,w	0.45 ± 0.01a,w
	II	0.49 ± 0.01	0.55 ± 0.01b,w	0.57 ± 0.02b,w	0.55 ± 0.01b,w	0.53 ± 0.02a,w	0.54 ± 0.01a,w	0.54 ± 0.01a,w	0.55 ± 0.01b,w	0.55 ± 0.01a,w	0.58 ± 0.01c,x
hexyl acetate	I	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	II	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
C6 from C18:2	I	0.65 ± 0.01	0.54 ± 0.01a,w	0.53 ± 0.01a,w	0.59 ± 0.02a,x	0.59 ± 0.01b,y	0.50 ± 0.03a,w	0.56 ± 0.01a,x	0.53 ± 0.01a,w	0.52 ± 0.01a,w	0.58 ± 0.02a,x
	II	0.71 ± 0.01	0.67 ± 0.01a,w	0.70 ± 0.01b,x	0.69 ± 0.01b,x	0.67 ± 0.01a,w	0.66 ± 0.01a,w	0.67 ± 0.02a,w	0.67 ± 0.01a,x	0.64 ± 0.01a,w	0.70 ± 0.01b,x
E-2-hexenal	I	1.96 ± 0.01	1.46 ± 0.01a,w	1.48 ± 0.02a,w	1.47 ± 0.02a,w	1.53 ± 0.01a,w	1.55 ± 0.02b,w	1.51 ± 0.01a,w	1.60 ± 0.01b,x	1.59 ± 0.01b,x	1.53 ± 0.01b,w
	II	1.09 ± 0.01	0.51 ± 0.01a,x	0.51 ± 0.01a,x	0.46 ± 0.01a,w	0.56 ± 0.01b,w	0.57 ± 0.02b,w	0.56 ± 0.01b,w	0.75 ± 0.01c,x	0.73 ± 0.02c,x	0.72 ± 0.01c,w
Z-3-hexen-1-ol	I	0.38 ± 0.01	0.64 ± 0.02b,w	0.63 ± 0.01a,w	0.65 ± 0.01b,w	0.60 ± 0.01a,w	0.62 ± 0.02a,w	0.62 ± 0.01a,w	0.63 ± 0.01b,w	0.63 ± 0.01a,w	0.63 ± 0.01a,w
	II	0.46 ± 0.01	0.66 ± 0.01b,w	0.66 ± 0.01b,w	0.66 ± 0.01b,w	0.63 ± 0.01a,w	0.63 ± 0.01a,w	0.63 ± 0.01a,w	0.65 ± 0.01b,w	0.67 ± 0.01b,w	0.70 ± 0.01c,x
E-2-hexen-1-ol	I	0.02 ± 0.01	0.03 ± 0.01a,w	0.03 ± 0.01a,w	0.03 ± 0.01a,w	0.02 ± 0.01a,w	0.02 ± 0.01a,w	0.02 ± 0.01a,w	0.04 ± 0.01b,w	0.04 ± 0.01b,w	0.04 ± 0.01b,w
	II	0.03 ± 0.01	0.03 ± 0.01a,w	0.03 ± 0.01b,w	0.03 ± 0.01a,w	0.03 ± 0.01a,w	0.03 ± 0.01a,w	0.03 ± 0.01a,w	0.04 ± 0.01b,w	0.04 ± 0.01b,w	0.04 ± 0.01b,w
Z-3-hexenyl acetate	I	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	II	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
C6 from C18:3	I	2.26 ± 0.01	2.13 ± 0.01a,w	2.10 ± 0.03a,w	2.15 ± 0.02a,w	2.15 ± 0.01a,w	2.19 ± 0.03b,w	2.15 ± 0.01a,w	2.27 ± 0.01b,x	2.26 ± 0.01c,x	2.20 ± 0.01b,w
	II	1.58 ± 0.01	1.19 ± 0.01a,x	1.19 ± 0.02a,x	1.14 ± 0.01a,w	1.22 ± 0.01a,w	1.22 ± 0.01a,w	1.22 ± 0.02a,w	1.43 ± 0.01b,w	1.44 ± 0.01b,w	1.46 ± 0.01b,x

[a] Different letters within a column (a−c) indicate significant differences ($p < 0.05$) with respect to malaxation time in olive paste. Different letters within a column (w-y) indicate significant differences ($p < 0.05$) with respect to olive paste at different stages of malaxation process.

Table 5. Effect of Malaxation Temperature on LOX Volatiles (Milligrams per Kilogram) in the Olive Paste (Median Kneading Time = 60 min)[a]

		20 °C				28 °C			40 °C		
		time = 0	47 min (P_{25})	60 min(P_{50})	73 min (P_{75})	47 min (P_{25})	60 min (P_{50})	73 min (P_{75})	47 min (P_{25})	60 min (P_{50})	73 min (P_{75})
hexanal	I	0.32 ± 0.02	0.19 ± 0.01b,w	0.16 ± 0.02b,w	0.26 ± 0.03c,x	0.06 ± 0.01a,w	0.04 ± 0.02a,w	0.10 ± 0.01b,x	0.05 ± 0.01a,w	0.05 ± 0.02a,w	0.07 ± 0.01a,x
	II	0.22 ± 0.01	0.16 ± 0.02b,x	0.13 ± 0.01b,w	0.10 ± 0.02b,w	0.14 ± 0.01b,w	0.12 ± 0.01b,w	0.14 ± 0.01c,w	0.02 ± 0.01a,w	0.01 ± 0.01a,w	0.02 ± 0.01a,w
hexan-1-ol	I	0.35 ± 0.01	0.44 ± 0.02b,w	0.43 ± 0.01b,w	0.46 ± 0.01b,w	0.46 ± 0.01b,w	0.47 ± 0.02c,w	0.46 ± 0.01b,w	0.40 ± 0.02a,w	0.40 ± 0.01a,w	0.39 ± 0.01a,w
	II	0.49 ± 0.01	0.57 ± 0.01b,w	0.58 ± 0.01b,w	0.57 ± 0.02a,w	0.53 ± 0.02a,w	0.54 ± 0.01a,w	0.54 ± 0.01a,w	0.61 ± 0.01c,w	0.59 ± 0.02b,w	0.61 ± 0.03b,w
hexyl acetate	I	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	II	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
C6 from C18:2	I	0.65 ± 0.01	0.63 ± 0.01c,x	0.58 ± 0.01c,w	0.71 ± 0.02c,y	0.59 ± 0.01b,y	0.50 ± 0.03b,w	0.56 ± 0.01b,x	0.45 ± 0.01a,w	0.44 ± 0.02a,w	0.46 ± 0.01a,w
	II	0.71 ± 0.01	0.72 ± 0.02c,x	0.70 ± 0.01c,x	0.66 ± 0.01b,w	0.67 ± 0.01b,w	0.66 ± 0.01b,w	0.67 ± 0.02b,w	0.63 ± 0.01a,w	0.59 ± 0.03a,w	0.63 ± 0.01a,w
E-2-hexenal	I	1.96 ± 0.01	2.06 ± 0.01c,w	2.05 ± 0.02c,w	2.03 ± 0.01c,w	1.53 ± 0.01b,w	1.55 ± 0.02b,w	1.51 ± 0.01b,w	1.45 ± 0.01a,w	1.46 ± 0.01a,w	1.43 ± 0.03a,w
	II	1.09 ± 0.01	0.66 ± 0.01c,w	0.69 ± 0.01c,w	0.68 ± 0.02c,w	0.56 ± 0.01b,w	0.57 ± 0.02b,w	0.56 ± 0.01b,w	0.39 ± 0.03a,w	0.36 ± 0.01a,w	0.38 ± 0.01a,w
Z-3-hexen-1-ol	I	0.38 ± 0.01	0.58 ± 0.02b,w	0.60 ± 0.01b,w	0.62 ± 0.01b,w	0.60 ± 0.01b,w	0.62 ± 0.02c,w	0.62 ± 0.01b,w	0.44 ± 0.01a,w	0.44 ± 0.01a,w	0.43 ± 0.03a,w
	II	0.46 ± 0.01	0.67 ± 0.01b,w	0.68 ± 0.02b,w	0.69 ± 0.01b,w	0.63 ± 0.01a,w	0.63 ± 0.01a,w	0.63 ± 0.01a,w	0.73 ± 0.03c,w	0.71 ± 0.02b,w	0.75 ± 0.01c,x
E-2-hexen-1-ol	I	0.02 ± 0.01	0.03 ± 0.01b,w	0.03 ± 0.01a,w	0.03 ± 0.01b,w	0.02 ± 0.01a,w	0.02 ± 0.01a,w	0.02 ± 0.01a,w	0.02 ± 0.01a,w	0.02 ± 0.01a,w	0.02 ± 0.01a,w
	II	0.03 ± 0.01	0.03 ± 0.01a,w	0.03 ± 0.01a,w	0.03 ± 0.01a,w	0.03 ± 0.01a,w	0.03 ± 0.01a,w	0.03 ± 0.01a,w	0.04 ± 0.01b,w	0.04 ± 0.01a,w	0.04 ± 0.01a,w
Z-3-hexenyl acetate	I	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	II	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
C6 from C18:3	I	2.26 ± 0.01	2.87 ± 0.02c,x	2.66 ± 0.03c,w	2.67 ± 0.01c,w	2.15 ± 0.01b,w	2.19 ± 0.03b,w	2.15 ± 0.01b,w	1.91 ± 0.01a,w	1.92 ± 0.01a,w	1.90 ± 0.02a,w
	II	1.58 ± 0.01	1.36 ± 0.02c,w	1.40 ± 0.03c,w	1.40 ± 0.01c,w	1.22 ± 0.01b,w	1.22 ± 0.01b,w	1.22 ± 0.02b,w	1.16 ± 0.01a,x	1.11 ± 0.03a,w	1.16 ± 0.01a,x

[a] Different letters within a column (a−c) indicate significant differences ($p < 0.05$) with respect to malaxation temperature in olive paste. Different letters within a column (w−y) indicate significant differences ($p < 0.05$) with respect to olive paste at different stages of malaxation process.

acetate and *Z*-3-hexyl-acetate (with sweet, fruity, and green leaf sensory notes), were present in very small amounts in the Cornicabra olive paste (**Tables 4** and **5**), showing the low activity of the alcohol acyl transferase (AAT) in this variety, as previously reported (*33*).

As for the effect of malaxation temperature on the C6 volatile compounds of olive paste (**Table 5**), their reduction as kneading temperature increased, especially in the cases of *E*-2-hexenal (from 2.05 to 1.46 mg/kg and from 0.69 to 0.36 mg/kg from 20 to 40 °C at kneading time P_{50} in the two batches studied, respectively) and hexanal (from 0.15 to 0.03 mg/kg from 20 to 40 °C, on average, for batches I and II), indicated that a lower temperature allowed higher activity of the LOX pathway enzymes, especially hydroperoxide lyase (HPL), which is known to possess the highest activity at 15 °C and shows large reductions at temperatures up 35 °C (*34*). Therefore, their content was greater at temperatures close to 20 °C. Almost no difference was observed in C6 alcohols (**Table 5**).

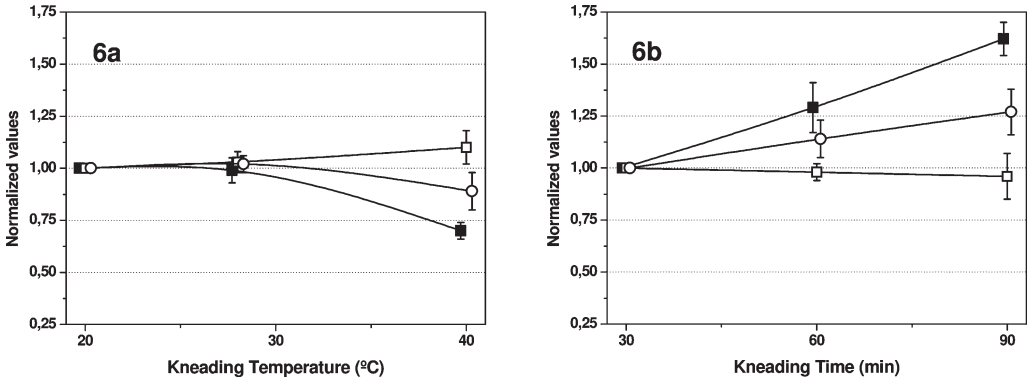

Figure 6. Effect of malaxation temperature (**a**) and time (**b**) on oil mill plant VOO volatile profile: ■, C6 aldehydes; □, C6 alcohols; ○, LOX volatiles (C6 volatiles + C5 volatiles). Normalized data are plotted with respect to those obtained at 20 °C/60 min (**a**) and at 30 min/28 °C (**b**), on average, for the two batches.

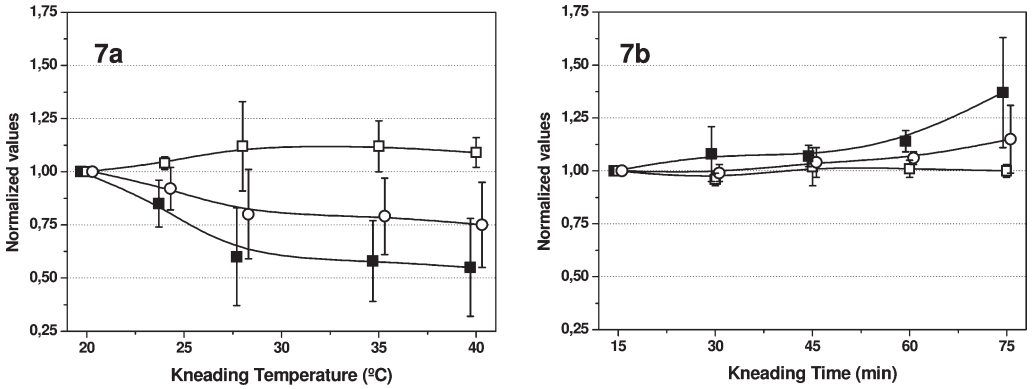

Figure 7. Effect of malaxation temperature (**a**) and time (**b**) on Abencor VOO volatile profile: ■, C6 aldehydes; □, C6 alcohols; ○, LOX volatiles (C6 volatiles + C5 volatiles). Normalized data are plotted with respect to those obtained at 20 °C/60 min (**a**) and at 30 min/28 °C (**b**), on average for the two batches.

Effect on Virgin Olive Oil Volatiles. After crushing of the olive fruits, the volatile content in the final VOO mainly depends on the partition phenomena of these compounds between the oily and water phases of the olive paste as well as the atmosphere (volatility), effects that are promoted by the malaxation process (*35*). Hence, a significant increase in C6 aldehydes was observed in the final oil as the malaxation time increased, chiefly the *E*-2-hexenal content, which rose by 70% on average for batches I and II between 30 and 90 min of kneading in the oil mill plant (**Figure 6b**); longer kneading times probably allow the volatile enhancement of the oily phase found in the paste. This is consistent with the findings of Angerosa et al. (*10*) and Ranalli et al. (*11*). Almost no changes in the content of C6 alcohols were found.

The influence of kneading temperature on volatile compounds from the LOX pathway in the virgin olive oils obtained in this study is depicted in **Figure 6a**, in which a trend similar to that described in the olive paste can be observed. In all cases a drop in the C6 aldehydes as the malaxation temperature increased was observed, especially in the *E*-2-hexenal content (30% reduction, on average, for batches I and II), as previously reported by Ranalli et al. (*8*). A very slight increase in C6 alcohols, mainly hexan-1-ol and *Z*-3-hexen-1-ol, was found at the higher temperature in the oils processed in the oil mill plant.

Similar trends in the volatile content in VOO processed under different malaxation temperature and time conditions at the laboratory scale using the Abencor apparatus were found (**Figure 7**), especially in terms of *E*-2-hexenal and hexanal, which decreased by an average of 48 and 32%, respectively, when the kneading temperature was increased from 20 to 40 °C. As mentioned in the case of the phenolic compounds, the use of a higher number of experimental malaxation temperatures and times allowed better observation of the behavior of the volatile components during this operation. Once again, the relevant effect of temperature was much greater from 20 to 28 °C, whereas in the case of malaxation time the major effect was observed at the higher kneading time.

In conclusion, the optimal malaxation conditions in the case of the Cornicabra cultivar, according to the results of this study, are a kneading temperature below 28 °C and a time greater than 60 min. In fact, for phenol-rich varieties such as this one (or cv. Picual and Coratina), a reduction in the phenolic content of the final virgin olive oil would improve the sensory properties of the product by means of a desirable decrease in its sensory bitterness, without significantly affecting its oxidative stability and shelf life. Moreover, the volatiles from the LOX pathway are favored by these kneading conditions, and therefore the green odor notes of the oil would increase. On the contrary, in the case of phenol-poor cultivars such as the Spanish Arbequina or Morisca and the Italian Leccino and Taggiasca, a further reduction of their naturally occurring antioxidants is of course not advisible and, therefore, a compromise between increasing biophenols and reducing volatiles should be established in each case.

LITERATURE CITED

(1) Angerosa, F. Influence of volatile compounds on virgin olive oil quality evaluated by analytical approaches and sensor panels *J. Lipid Sci. Technol.* **2002**, *104*, 639–660.
(2) Aparicio, R.; Luna, G. Characterisation of monovarietal virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 614–627.
(3) Sanchez, J.; Harwood, J. L. Byosinthesis of triacylglycerols and volatiles in olives. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 564–573.
(4) Angerosa, F.; Mostallino, R.; Basti, C.; Vito, R. Virgin olive oil odour notes: their relationship with volatile compounds from the

lipoxygenase pathway and secoiridoid compounds. *Food Chem.* **2000**, *68*, 283–287.
(5) Caponio, F.; Gomes, T.; Summo, C.; Pasqualone, A. Influence of the type of olive-crusher used on the quality of extra virgin olive oils. *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 201–206.
(6) Di Giovachino, L.; Sestili, S.; Di Vincenzo, D. Influence of olive processing on virgin olive oil quality. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 587–601.
(7) Servili, M.; Piacquadio, P.; De Stefano, G.; Taticchi, A.; Sciancalepore, V. Influence of a new crushing technique on the composition of the volatile compounds and related sensory quality of virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 483–489.
(8) Ranalli, A.; Contento, S.; Schiavone, C.; Simone, N. Malaxing temperature affects volatile and phenol composition as well as other analytical features of virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2001**, *103*, 228–238.
(9) Jiménez Márquez, A.; Hermoso Fernández, M.; Uceda Ojeda, M. Elaboración del aceite de oliva virgen mediante sistema continuo en dos fases. Influencia de diferentes variables del proceso en algunos parámetros relacionados con la calidad del aceite. *Grasas Aceites* **1995**, *46*, 299–303.
(10) Angerosa, F.; Mostallino, R.; Basti, C.; Vito, R. Influence of malaxation temperature and time on the quality of virgin olive oils. *Food Chem.* **2001**, *72*, 19–28.
(11) Ranalli, A.; Pollastri, L.; Contento, S.; Lannucci, E.; Lucera, L. Effect of paste kneading process time on the overall quality of virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 57–67.
(12) Lercker, G.; Frega, N.; Bocci, F; Mozzori, M. Volatile constituents and oxidative stability of virgin olive oils: influence of the kneading of the olive paste. *Grasas Aceites* **1999**, *50*, 26–29.
(13) Servilli, M.; Selvaggini, R.; Taticchi, A.; Esposto, S.; Montedoro, G. Air exposure time of olive pastes during the extraction process and phenolic and volatile composition of virgin olive oil. *J. Am. Oil Chem. Soc.* **2003**, *80*, 685–695.
(14) Kalua, C. M.; Bedgood, D. R.; Bishop, A. G.; Prenzler, P. D. Changes in volatile and phenolic compounds with malaxation time and temperature during virgin olive oil production. *J. Agric. Food Chem.* **2006**, *54*, 7641–7651.
(15) UNE Spanish Standard (Asociación Española de Normalización y Certificación). UNE 55032: Fats. Determination of oil grease in total fat matter (Materias grasas. Determinación del contenido en materia grasa total del orujo de aceituna); AENOR: Madrid, Spain, 1973.
(16) Vichi, S.; Castellote, A. I.; Pizzale, L.; Conte, L. S.; Buxaderas, S.; Lopez-Tamames, E. Analysis of virgin olive oil volatile compounds by headspace solid-phase microextraction coupled to gas chromatography with mass spectrometric and flame ionisation detection *J. Chromatogr., A* **2003**, *983*, 19–33.
(17) Mateos, R.; Espartero, J. L.; Trujillo, M.; Ríos, J. J.; León-Camacho, M.; Alcudia, F.; Cert, A. Determination of phenols, flavones and lignans in virgin olive oil by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection. *J. Agric. Food Chem.* **2001**, *49*, 2185–2192.
(18) Impellizzeri, J.; Jianming, L. A simple high-performance liquid chromatography method for the determination of throat-burning oleocanthal with probated antiinflammatory activity in extra virgin olive oils. *J. Agric. Food Chem.* **2006**, *54*, 3204–3208.
(19) Inarejos-García, A. M.; Gómez-Rico, A.; Salvador, M. D.; Fregapane, G. Influence of malaxation conditions on virgin olive oil yield, overall quality and composition. *Eur. Food Res. Technol.* **2009**, *228*, 671–677.
(20) Amiot, M. J.; Fleuriet, A.; Macheix, J. J. Importance and evolution of phenolic compounds in olive during growth and maturation *J. Agric. Food Chem.* **1986**, *34*, 823–826.
(21) Amiot, M. J.; Fleuriet, A.; Macheix, J. J. Accumulation of oleuropein derivatives during olive maturation. *Phytochemistry* **1989**, *28*, 67–69.
(22) Servili, M.; Baldioli, M.; Selvaggini, R.; Macchioni, A.; Montedoro, G. F. Phenolic compounds of olive fruit: one- and two-dimensional nuclear magnetic resonance characterization of nüzhenide and its distribution in the constituve parts of fruit. *J. Agric. Food Chem.* **1999**, *47*, 12–18.
(23) Ryan, D.; Antolovich, M.; Prenzler, P.; Robards, K.; Lavee, S. Biotransformations of phenolic compounds in *Olea europaea* L. *Sci. Hortic.* **2002**, *92*, 147–176.
(24) Artajo, L. S.; Romero, M. P.; Suarez, M.; Motilva, M. J. Partition of phenolic compounds during the virgin olive oil industrial extraction process. *Eur. Food Res. Technol.* **2006**, *225*, 617–625.
(25) Servili, M.; Baldioli, M.; Begliomini, A.; Selvaggini, R.; Montedoro, G. F. The phenolic and volatile compounds of virgin olive oil: relationship with the endogenous oxidoreductases during the mechanical oil extraction process. In Flavour and Fragrance Chemistry; Proceedings of the Phytochemical Society of Europe, Campobasso, Italy, January 13−16 2000; Kluwer Academic: Dordrecht, The Netherlands, 2000; pp 163−173.
(26) Toscano, G.; Colarieti, M. L.; Greco, G. Oxidative polymerisation of phenols by a phenol oxidase from green olives. *Enzyme Microb. Technol.* **2003**, *33*, 47–54.
(27) Rodis, P. S.; Karathanos, V. T.; Mantzavinou, A. Partitioning of olive oil antioxidants between oil and water phases. *J. Agric. Food Chem.* **2002**, *50*, 596–601.
(28) Di Giovacchino, L. L'estrazione dell'olio con la centrifugazione diretta delle paste di oliva. Nota I: influenza della gramolazione. *Riv. Ital. Sostanze Grasse* **1991**, *68*, 314–420.
(29) Servili, M.; Baldioli, G.; Montedoro, G. Phenolic composition of virgin olive oil in relationship to some chemical and physical aspects of malaxation. *Acta Hortic.* **1994**, *356*, 331–336.
(30) Andrewes, P.; Busch, J.; De Joode, T.; Groenewegen, A.; Alexandre, H. Sensory properties of virgin olive oil polyphenols: identification of deacetoxy-ligstroside aglycone as a key contributor to pungency. *J. Agric. Food Chem.* **2003**, *51*, 1415–1420.
(31) Beauchamp, G. K.; Keast, R. S. J.; Morel, D.; Lin, J.; Pika, J.; Han, Q.; Lee, C.-H.; Smith, A. B.; Breslin, P. A. S. Ibuprofen-like activity in extra virgin olive oil: enzymes in an inflammation pathway are inhibited by oleocanthal, a component of olive oil. *Nature (London)* **2005**, *437*, 45–46.
(32) Gutiérrez-Rosales, F.; Rios, J. J.; Gomez-Rey, M. L. Main polyphenols in the bitter taste of virgin olive oil. Structural confirmation by on-line high-performance liquid chromatography electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 6021–6025.
(33) Gómez-Rico, A.; Salvador, M. D.; La Greca, M.; Fregapane, G. Phenolic and volatile compounds of extra virgin olive oil (*Olea europaea* L. Cv. Cornicabra) with regard to fruit ripening and irrigation management. *J. Agric. Food Chem.* **2006**, *54* 7130–7136.
(34) Salas, J. J.; Sanchez, J. The decrease of virgin olive oil flavour produced by high malaxation temperature is due to inactivation of hydroperoxide lyase. *J. Agric. Food Chem.* **1999**, *47*, 809–812.
(35) Angerosa, F.; d'Alessandro, N.; Basti, C.; Vito, R. Biogeneration of volatile compounds in virgin olive oil: their evolution in relation to malaxation time. *J. Agric. Food Chem.* **1998**, *46*, 2940–2944.

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