Eur Food Res Technol (2009) 228:671–677
DOI 10.1007/s00217-008-0977-9

ORIGINAL PAPER

Influence of malaxation conditions on virgin olive oil yield, overall quality and composition

Antonio M. Inarejos-García · Aurora Gómez-Rico · M. Desamparados Salvador · Giuseppe Fregapane

Received: 9 June 2008 / Revised: 27 August 2008 / Accepted: 10 October 2008 / Published online: 13 November 2008

Abstract The production of a high quality product requires good manufacturing practices and effective quality assurance throughout the whole process. The aim of this study was to improve the knowledge of the technological variables involved in the malaxation operation, which affect the yield and in particular the overall quality of the virgin olive oil. A continuous experimental virgin olive oil mill plant (Pieralisi, Fattoria) was employed, working at 200 kg/h olive paste, and the paste was kneaded at different temperatures (20–40 °C) and times (30–90 min). The industrial oil yield improved when either the kneading temperature or the kneading time was increased, but the temperature affected more the oil yield than the time (yield increased by about 28%, from 20 to 40 °C). The total phenol and *o*-diphenol contents in virgin olive oils, which affect both the sensory bitterness and the oxidative stability of the oils, greatly improved with the increase in the malaxation temperature. On the contrary, a longer kneading time only led to a slight decrease in the total phenols. This fact is very important since excessive levels of phenols, typical of Cornicabra virgin olive oils, may adversely affect consumer preference for this highly valued product.

Keywords Olive oil · Malaxation · Oil mill plant · Yield · Composition · Quality · Bitterness

A. M. Inarejos-García · A. Gómez-Rico · G. Fregapane (✉)
Departamento de Tecnología de los Alimentos,
Universidad de Castilla-La Mancha, Ciudad Real, Spain
e-mail: giuseppe.fregapane@uclm.es

M. D. Salvador
Instituto Regional de Investigación Científica Aplicada (IRICA),
Universidad de Castilla-La Mancha, Ciudad Real, Spain

Introduction

Virgin olive oil is obtained from the fruit of the olive tree (*Olea europaea* L.) using only mechanical processes. The juice of this fruit is an oil that is ready for human consumption and possesses unique sensory characteristics and nutritional properties. This vegetable oil is the main edible fat source used in the Mediterranean area; and moreover, its consumption has increased in other countries in recent years [1].

The production of a high quality virgin olive oil requires healthy olive fruit, good manufacturing practices and effective quality assurance throughout the entire manufacturing process, including proper storage of the virgin olive oil that is produced. Several researchers have noted that the technological operations that most affect virgin olive oil quality and composition during the manufacturing process are milling and malaxation.

For instance, mechanical crushers extract more phenols from the vegetable tissues than the traditional stone mills [2], so that the oils possess higher antioxidant capacity [3]. Servili et al. [4] reported changes also in the volatile composition and pigment content of the oil depending on the type of mill employed.

It is worth stressing the importance of the olive paste malaxation process; kneading is in fact much more than a simple physical separation; a complex bioprocess takes place, which very much affects the final product quality and composition. Indeed malaxation conditions have been shown to affect not only the oil yield but in particular the composition and quality of the final virgin olive oil. For example, increasing the kneading temperature generally improves phenol contents [5, 6] with a consequent improvement in oxidative stability [7], whereas volatile content usually decreases under these conditions [6]. On the

contrary, a longer kneading time apparently affects the phenol contents negatively, while increasing the presence of oil volatiles [8–12]. Nevertheless, these technological studies did not generally focus on the enhancement of the overall quality of the product or on the effect of the malaxation conditions on olive oil bitterness.

Moreover, in the particular case of the Cornicabra variety a monovarietal virgin olive oil of great economic importance in Castilla-La Mancha (Spain), which is characterised by high oxidative stability and intense bitterness; thorough research will be required to arrive at malaxation conditions that achieve a satisfactory compromise between improvement of oil yield and improvement of oil quality.

The aim of this study was therefore to learn more in the scientific and technological terms about the variables involved in the kneading operation, which affect both the yield and the overall quality and composition of the Cornicabra virgin olive oil performed by the industrial oil mills in our region. The right combination of temperature and time conditions during malaxation needs to be used in order in particular to enhance the sensory characteristics and hence consumer acceptance of the oil.

To this end, the authors used a continuous experimental virgin olive oil mill plant (Pieralisi, Fattoria). The olive paste was kneaded at different temperatures (20–40 °C) and times (30–90 min) both in the pilot plant and on laboratory scale equipment (Abencor), producing a wide matrix of results covering the range of real malaxation conditions used by the industry. In order to study the influence of malaxation, oil yield mass balance data, quality indices (e.g. free acid content, peroxide index, K_{232}, K_{270}, waxes, bitter index), antioxidants and relevant minor nutritional compounds (e.g. phenolic compounds, α-tocopherol, *β*-carotene), oxidative stability and antiradical capacity (Rancimat, DPPH method) were analysed and their observed experimental data discussed.

Materials and methods

Experimental oil mill plant

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.

Virgin olive oil samples

Two different batches of Cornicabra cv. fruit were used, each weighing approximately 1,600 kg. The malaxation assays were performed at different temperature (20, 28 and 40 °C) and time (30, 60 and 90 min) conditions; batches of 200 kg of fruit were processed in each assay. Also, different assays were performed on the same olive batches using the laboratory scale Abencor system, in order to amplify with the range of temperature–time conditions studied (20, 24, 28, 35 and 40 °C and 15, 30, 45, 60 and 75 min). All samples were filtered with anhydrous Na_2SO_4 and stored at 4 °C in darkness using amber glass bottles without head space prior to analysis.

Analytical determinations in olive fruits and olive pomace

The olive ripeness index was determined according to the method proposed by the International Olive Oil Council [13], based on the evaluation of the olive skin and pulp colours. Ripeness index values range from 0 (100% intense green skin) to 7 (100% purple flesh and black skin).

The water content of olive fruit and olive pomace was determined by desiccation according to the UNE Spanish Standard method 55032:1973 [14]. The fat content was determined by Soxhlet extraction and was expressed as a percentage of dry olive paste weight [14].

Analytical determinations in virgin olive oil

Free acidity, given as percentage of oleic acid, peroxide value (PV) expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2/kg), K_{232} and K_{270} extinction coefficients calculated from absorption at 232 and 270 nm and *waxes content*, given as mg per kilogram of oil (mg/kg), were measured following the analytical methods described in [15] and subsequent amendments.

Fatty acid composition, according to European Regulations and subsequent amendments, corresponding to the AOCS method Ch 2–91 [15]. To determine fatty acid composition, the methyl-esters were prepared by vigorous shaking of a solution of oil in hexane (0.2 g in 3 ml) with 0.4 ml of 2 mol/l methanolic potassium hydroxide and analysed by GC with a FID detector. A fused silica column (50 m length × 0.25 mm i.d.) coated with SGL-1000 phase (0.25 μm thickness; Sugerlabor, Spain) was used. The carrier gas was helium, at a flow through the column of 1 ml/min. The injector and detector temperature was set at 250 °C and the oven temperature at 210 °C. The injection volume was 1 μl.

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., Bellefonte, USA) was used to extract the phenolic fraction. The cartridge was conditioned first with methanol (6 ml)

and then with 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). HPLC analysis was performed 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 i.d. mm, 5 µm particle size) (Agilent Technologies, USA) was used, maintained at 30 °C, with an injection volume of 20 µl and a flow rate of 1.0 ml/min. 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. Phenolic compounds were quantified at 280 nm using syringic acid as internal standard and the response factors determined by Mateos et al. [16].

Tocopherols were evaluated following AOCS method Ce8-89 [17]. A solution of oil in hexane was analysed on an Agilent Technologies HPLC (1100 series) on a silica gel Lichrosorb Si-60 column (particle size 5 µm, 250 mm × 4.6 mm i.d.; Sugerlabor, Madrid, Spain) which was eluted with hexane/2-propanol (98.5:1.5) at a flow rate of 1 ml/min. A fluorescence detector (Thermo-Finnigan FL3000) was used with excitation and emission wavelength set at 290 and 330 nm.

β-carotene content was evaluated following the method described by Gimeno et al. [18]. A sample of olive oil (400 mg) was weighed in a centrifugation tube and then 0.2 g of ascorbic acid, 15 ml of absolute ethanol and 4 ml of 76% potassium hydroxide solution were added under a stream of nitrogen. The tubes were incubated at 70 °C for 30 min with slow constant stirring. Sodium chloride (5 ml, 25 g/l) was added and the suspension was extracted three times with *n*-hexane-ethyl acetate (15 ml, 85:15, v/v). The organic phase was evaporated to dryness at 40 °C and the residue was dissolved in 0.5 ml of methanol. HPLC analysis was performed using the same equipment previously described for the determination of the phenolic compounds, a ZORBAX SB-C18 column (250 × 3.0 i.d. mm, 5 µm particle size), maintained at 45 °C, with an injection volume of 25 µl and a flow rate of 1.25 ml/min. Mobile phase was a mixture of methanol (solvent A), milli-Q water (B) and butanol (C): from 92% (A)–3% (B)–5% (C) for 5 min to 92% (A)–8% (C) in 1 min and maintained for 6 min, then the system returned to the initial conditions. *β*-carotene was quantified at 450 nm.

DPPH radical scavenging effect. DPPH was employed as a stable radical [19]. Several concentrations of SPE phenolic extracts dissolved in methanol (0.1 ml) were added to a DPPH methanolic solution (2.9 ml, 6.0×10^{-5} mol/l). The decrease in absorbance of the resulting solution was then measured at 515 nm at 0, 5, 10, 15 min, and then every 15 min until 1 h. The absorbance was plotted against time, and the percentage of absorbance reduction at 15 min was used as a measure of the antioxidant activity of the extract. A calibration curve was determined using Trolox as an external standard with a range of concentrations from 0.19 to 0.93 meq/l. The results were expressed as miliequivalents of Trolox per kilogram of virgin olive oil.

Oxidative stability. This was evaluated by the Rancimat method [20]. Stability was expressed as the induction time (hours) measured with the Rancimat 679 apparatus (Metrohm, Switzerland) at 120 °C and an air flow of 20 l/h.

Bitterness index (K_{225}) was determined by the method described by Gutierrez et al. [21], which consists of the extraction of the bitter components from a sample of 1.0 ± 0.01 g of oil dissolved in 4 ml of hexane passed through a C_{18} column (Bakerbond SPE, J.T. Baker, Phillipsburg, NJ, USA) previously activated with methanol and washed with hexane. After elution, 10 ml of hexane was passed to eliminate the oil residues and then the retained compounds were eluted with methanol/water (1:1) to 25 ml. The absorbance of the extract was measured at 225 nm against methanol/water (1:1) in a 1 cm cuvette.

All experiments and analytical determinations were carried out at least in duplicate. All reagents used were of analytical, HPLC or spectroscopic grade, and were supplied by Merck (Darmstadt, Germany). α-Tocopherol, *β*-carotene, syringic acid, ascorbic acid, DPPH (2,2-difenyl-1-picryl hydracyl), Trolox ((±)-hydroxy-2,5,7,8-tetra-methyl-chromane-2-carboxylic acid) reagents were purchased from Sigma-Aldrich (Steinheim, Germany).

Statistical analysis

Statistical analyses were performed using SPSS 14.0 statistical software (SPSS Inc., Chicago, IL).

Results and discussion

The two batches of Cornicabra olive fruit employed in this assay presented similar values for ripeness index, oil content, humidity and dry matter as reported in Table 1.

Oil yield

During the olive oil processing carried out in the experimental oil mill plant the composition of the olive fruit and olive pomace was determined in order to describe the mass

Table 1 Initial composition of Cornicabra cv. olive fruit batches employed in the technological assays

Batch	Ripeness index	Oil yield (% fresh weight)	Humidity (%)	Oil yield (% dry weight)	Dry extract (%)
I	4.5	24.6	40.3	41.2	35.4
II	4.7	28.4	37.5	45.4	34.1

balance of the process and study the effect of malaxation temperature–time conditions on the oil yield (Tables 2, 3). In this process there is one input, "olive fruit" raw material, and two output products, "virgin olive oil" and a subproduct "olive pomace".

As expected, the mean value of the industrial oil yield improved when either the kneading temperature or time was increased. However, the temperature effect on the oil yield, which rose by 28% when this parameter was increased from 20 to 40 °C at a fixed malaxation time of 60 min (Fig. 1a, b), was superior than when the kneading time was prolonged, probably because the oil became less viscous and hence more readily extracted from the vegetable tissue [3, 5, 6]. The behaviour of samples was similar in the oil mill plant and the laboratory scale Abencor; note however that the oil yield increase was significantly higher in the oil mill, especially at longer malaxation times (Fig. 1b), probably due to the formation of oil–water emulsions [11, 12] in the vessel used by the Abencor system which is much smaller than the oil mill kneader.

As expected, the oil content of the olive pomace produced in the different technological assays decreased as kneading temperature and time increased (Tables 2, 3).

Virgin olive oil quality and composition

Quality indices

It is very important to know the effect of the malaxation conditions on the quality of the virgin olive oil produced in order to strike an appropriate balance between the economic turnover and the quality of the commercial product.

All the values of the quality indices in the different malaxation conditions studied were well below the upper limits established by EU Regulations for the extra virgin olive oil category [22]. However, they increased, very slightly but steadily, when either the kneading temperature (Table 4) or the kneading time (Table 5) was increased. This was probably due to augmented lipase enzyme activity (responsible for increasing free acidity) and to an increase in the oxidation rate (responsible for the increase of peroxide value, K_{232}

Table 2 Mass balance as affected by malaxation temperature (20–40 °C) in the oil mill plant

Malaxation temperature (°C)		Fruit	Olive oil		Pomace			
		Weight (kg)	Weight (kg)	Oil yield (%)	Weight (kg)	Oil (%)	Water (%)	Dry extract (%)
20	I	196.0	35.9	18.3	160.1	9.0	48.3	42.7
	II	216.0	35.9	16.6	180.1	13.2	44.6	42.1
28	I	199.0	41.0	20.6	158.0	6.7	50.5	42.9
	II	206.0	39.1	19.0	166.9	12.2	45.1	42.7
40	I	216.0	45.2	20.9	170.8	6.0	51.0	43.1
	II	203.0	47.8	23.5	155.2	7.9	46.6	45.5

Malaxation time constant: 60 min

Table 3 Mass balance as affected by malaxation time (30–90 min) in the oil mill plant

Malaxation time (min)		Fruit	Olive oil		Pomace			
		Weight (kg)	Weight (kg)	Oil yield (%)	Weight (kg)	Oil (%)	Water (%)	Dry extract (%)
30	I	225.0	45.7	20.3	180.3	5.8	52.1	42.1
	II	231.0	40.2	17.4	191.8	13.8	43.2	43.0
60	I	199.0	41.0	20.6	158.0	6.7	50.5	42.9
	II	206.0	39.1	19.0	166.9	12.2	45.1	42.7
90	I	209.0	43.9	21.0	166.1	4.8	50.6	44.6
	II	236.0	47.4	20.1	189.6	11.0	44.9	44.1

Malaxation temperature constant: 28 °C

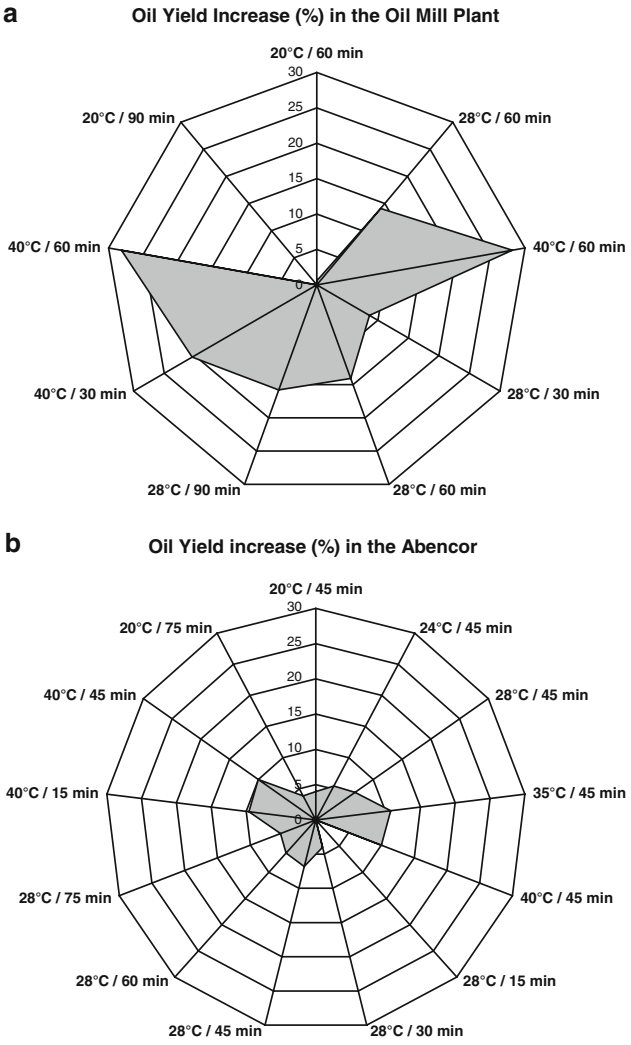

Fig. 1 Increase in oil yield as affected by kneading temperature and time in the Oil mill plant (**a**) and in the laboratory scale Abencor equipment (**b**)

and K_{270} values) [11, 23]. Nevertheless there were no statistically significant differences in the free acidity, peroxide value and ultraviolet absorption characteristics in the different assays at either mill pilot plant or laboratory scale, even in the most drastic conditions 40 °C or 90 min of kneading. Similar results were reported in [6, 11, 23].

On the other hand, there was a statistically significant rise in wax content as the kneading time and temperature increased. However, the highest wax content observed in this study was 98.5 mg/kg, which is well short of the upper legal limit of 250 mg/kg established by EU regulations [22]. Waxes are produced by interaction between the olive oil's alcohols and free fatty acids. Increasing temperature favours the release of these components from the vegetal tissue because the waxes become more oil soluble [24].

Fatty acid composition

The fatty acid composition of the triacylglycerols was not influenced by either malaxation temperature or time conditions (data not shown), contrary to Ranalli et al. [6] who reported a reduction in polyunsaturated fatty acids due to oxidation as the kneading temperature increased.

Minor components of antioxidant, nutritional and sensory value

The total phenol, *o*-diphenol, α-tocopherol and *β*-carotene contents and the oxidative stability, antioxidant capacity (DPPH method) and bitterness index (K225) for the virgin olive oils produced by processing under the different kneading conditions are shown in Tables 6, 7.

As regards the antioxidant content of the virgin olive oil studied, it is important to note that total phenols and *o*-diphenols content increased considerably in virgin olive oils with increasing malaxation temperature (between 75 and 120% from 20 to 40 °C; Table 6). This was probably because a higher kneading temperature implied a rise in the partition coefficient of phenolic compounds between the oil and water phases of the olive paste, raising the phenolic content in the virgin olive oil obtained [25]. Our results were consistent with other studies on industrial olive oil mills [5–7, 26]. Other authors [9, 27], reported on the con-

Table 4 Virgin olive oil quality indices as affected by malaxation temperature (20–40 °C)

Malaxation temperature (°C)	Oil mill plant			Lab scale (Abencor)				
	20	28	40	20	24	28	35	40
Free acidity (%)	0.21 ± 0.01^{a}	0.21 ± 0.01^{a}	0.25 ± 0.03^{a}	0.25 ± 0.02^{a}	0.27 ± 0.01^{a}	0.28 ± 0.02^{a}	0.28 ± 0.01^{a}	0.29 ± 0.02^{a}
Peroxide value ($meqO_2/kg$)	5.9 ± 0.2^{a}	6.2 ± 0.01^{a}	6.8 ± 0.4^{a}	5.7 ± 0.1^{a}	5.9 ± 0.1^{a}	6.1 ± 0.4^{a}	6.2 ± 0.4^{a}	6.8 ± 0.8^{a}
K_{232}	1.60 ± 0.13^{a}	1.67 ± 0.17^{a}	1.70 ± 0.13^{a}	1.62 ± 0.08^{a}	1.65 ± 0.10^{a}	1.71 ± 0.11^{a}	1.73 ± 0.12^{a}	1.78 ± 0.09^{a}
K_{270}	0.14 ± 0.01^{a}	0.15 ± 0.02^{a}	0.16 ± 0.02^{a}	0.14 ± 0.02^{a}	0.15 ± 0.03^{a}	0.16 ± 0.04^{a}	0.16 ± 0.03^{a}	0.17 ± 0.03^{a}
Waxes (mg/kg IS)	76.5 ± 0.5^{a}	79.5 ± 0.7^{b}	85.5 ± 0.8^{c}	83.5 ± 0.6^{a}	89.5 ± 0.5^{b}	$90.5 \pm 0.7^{b,c}$	91.5 ± 0.6^{c}	98.5 ± 0.8^{d}

Malaxation time constant: 60 min for the experimental oil mill pilot plant; 45 min for the Abencor laboratory scale system

Different letters within a quality index (a–d) indicate significant differences ($p < 0.05$)

Table 5 Virgin olive oil quality indices as affected by malaxation time (15–90 min)

Malaxation time (min)	Oil mill plant			Lab scale (Abencor)				
	30	60	90	15	30	45	60	75
Free acidity (%)	0.21 ± 0.01^{a}	0.21 ± 0.01^{a}	0.22 ± 0.01^{a}	0.26 ± 0.02^{a}	0.27 ± 0.01^{a}	0.28 ± 0.02^{a}	0.28 ± 0.01^{a}	0.27 ± 0.01^{a}
Peroxide value ($meqO_2/kg$)	6.3 ± 0.1^{a}	6.2 ± 0.01^{a}	7.4 ± 0.2^{b}	6.2 ± 0.5^{a}	6.7 ± 0.5^{a}	6.1 ± 0.4^{a}	6.9 ± 0.7^{a}	7.2 ± 0.9^{a}
K_{232}	1.75 ± 0.11^{a}	1.67 ± 0.17^{a}	1.70 ± 0.12^{a}	1.79 ± 0.04^{a}	1.77 ± 0.07^{a}	1.71 ± 0.11^{a}	1.69 ± 0.04^{a}	1.68 ± 0.12^{a}
K_{270}	0.15 ± 0.02^{a}	0.15 ± 0.02^{a}	0.15 ± 0.03^{a}	0.17 ± 0.02^{a}	0.19 ± 0.00^{a}	0.16 ± 0.04^{a}	0.15 ± 0.03^{a}	0.15 ± 0.03^{a}
Waxes (mg/kg IS)	75.5 ± 0.5^{a}	79.5 ± 0.7^{b}	81.5 ± 0.8^{b}	90.5 ± 0.6^{a}	94.5 ± 0.5^{b}	90.5 ± 0.7^{a}	96.5 ± 0.6^{c}	97.5 ± 0.8^{c}

Malaxation temperature constant: 28 °C

Different letters within a quality index (a–d) indicate significant differences ($p < 0.05$)

Table 6 Virgin olive oil minor components and related properties as affected by malaxation temperature (20–40 °C)

Malaxation temperature (°C)		Oil mill plant			Lab scale (Abencor)				
		20	28	40	20	24	28	35	40
Total phenols (mg/kg)	I	450.5 ± 9.1^{a}	649.0 ± 7.0^{b}	788.5 ± 6.3^{c}	467.5 ± 6.3^{a}	516.5 ± 9.1^{b}	723.0 ± 5.6^{c}	832.5 ± 4.9^{d}	885.0 ± 9.5^{e}
	II	265.5 ± 5.5^{a}	408.8 ± 2.1^{b}	532.3 ± 8.1^{c}	333.3 ± 9.7^{a}	390.3 ± 4.2^{b}	481.2 ± 8.5^{c}	581.6 ± 8.7^{d}	672.7 ± 7.7^{e}
o-Diphenols (mg/kg)	I	205.5 ± 0.7^{a}	326.5 ± 2.5^{b}	390.5 ± 2.7^{c}	240.9 ± 11.0^{a}	253.0 ± 8.5^{a}	443.5 ± 2.1^{b}	500.0 ± 4.2^{c}	527.4 ± 9.0^{d}
	II	104.5 ± 9.9^{a}	183.7 ± 3.3^{b}	267.0 ± 9.5^{c}	141.1 ± 7.7^{a}	146.0 ± 2.1^{a}	225.4 ± 8.5^{b}	275.8 ± 9.7^{c}	343.8 ± 3.5^{d}
α-Tocopherol (mg/kg)	I	205 ± 3^{a}	219 ± 1^{b}	221 ± 2^{b}	207 ± 3^{b}	200 ± 2^{a}	194 ± 3^{a}	216 ± 3^{c}	225 ± 2^{d}
	II	186 ± 1^{a}	188 ± 3^{a}	193 ± 3^{a}	186 ± 4^{a}	197 ± 3^{b}	201 ± 2^{b}	186 ± 3^{a}	195 ± 2^{b}
β-Carotene (mg/kg)		0.63 ± 0.09^{a}	0.87 ± 0.10^{b}	1.56 ± 0.12^{c}	–	–	–	–	–
Oxidative stability (h)	I	14.7 ± 0.3^{a}	21.8 ± 0.2^{b}	24.7 ± 0.3^{c}	17.2 ± 0.2^{a}	18.7 ± 0.4^{b}	23.8 ± 0.3^{c}	26.8 ± 0.2^{d}	29.1 ± 0.2^{e}
	II	14.1 ± 0.2^{a}	18.3 ± 0.9^{b}	20.8 ± 0.2^{c}	16.8 ± 0.1^{b}	15.4 ± 0.5^{a}	19.4 ± 0.4^{c}	20.7 ± 0.3^{d}	23.6 ± 0.2^{e}
DPPH test (meq Trolox/kg)	I	947 ± 38^{a}	1520 ± 28^{b}	1739 ± 71^{c}	1258 ± 39^{a}	1433 ± 10^{b}	2124 ± 33^{c}	2368 ± 19^{d}	2484 ± 22^{e}
	II	589 ± 15^{a}	730 ± 42^{b}	1358 ± 2^{c}	826 ± 8^{b}	846 ± 9^{b}	744 ± 8^{a}	1374 ± 2^{c}	1539 ± 14^{d}
K_{225}	I	0.46 ± 0.01^{a}	0.52 ± 0.03^{b}	0.56 ± 0.2^{c}	0.45 ± 0.01^{a}	0.47 ± 0.02^{b}	0.57 ± 0.02^{c}	0.62 ± 0.01^{d}	0.64 ± 0.02^{e}
	II	0.42 ± 0.02^{a}	0.47 ± 0.01^{b}	0.54 ± 0.02^{c}	0.45 ± 0.01^{a}	0.46 ± 0.01^{a}	0.52 ± 0.02^{b}	0.52 ± 0.01^{b}	0.57 ± 0.02^{c}

Malaxation time constant: 60 min for the experimental oil mill pilot plant; 45 min for the Abencor laboratory scale system

Different letters within a component (a–d) indicate significant differences ($p < 0.05$)

trary that the phenol content decreased as the kneading temperature of the olive paste increased, although these experiments were carried out on small-scale laboratory equipment and not in pilot or industrial oil mill plants.

Increasing kneading time, on the other hand, had a little effect on phenolic composition (Table 7). In fact, it reduced the *o*-diphenol content only slightly and raised the total phenols only a little, to extents that were significantly different in statistical terms only in the oils processed in the Abencor system. The slight fall observed in *o*-diphenols (secoiridoid derivatives of the hydroxytyrosol) was probably due to enzymatic oxidation [8, 10, 11] and to more prolonged contact with the vegetation water, favouring the diffusion of these phenols to the aqueous phase [7]. Similar results have been observed in [7–12].

Of the other minor compounds of nutritional and antioxidant value, α-tocopherol (vitamin E) and *β*-carotene (provitamin A) contents were less affected than phenols by either malaxation time or temperature (Table 6, 7). However, their values increased slightly with malaxation temperature (Table 6), as also reported by Ranalli et al. [6], since higher temperature promotes the release of these compounds from the vegetable tissues of the fruit.

Oxidative stability and antiradical capacity, as measured by Rancimat and DPPH methods, respectively, changed according to the concentration of *o*-diphenols in particular and were therefore greatly improved by increasing the kneading temperature, with average increases of 50 and 100% in oxidative stability antiradical capacity, respectively (Table 6).

It is known that the intensity of sensory pungency and especially bitterness are related to the phenol content in the olive oil. Olive oil bitterness can also be measured by the instrumental K_{225} parameter called the bitterness index [21]. The evolution of this index is reported in Tables 6, 7. It is important, particularly as far as Cornicabra varieties are concerned, to note that the bitter index (K_{225}) increased with the temperature (about 25% from 20 to 40 °C) and decreased with kneading time (about 15% from 30 to

Table 7 Virgin olive oil minor components and related properties as affected by malaxation time (15–90 min)

Malaxation time (min)		Oil mill plant			Lab scale (Abencor)				
		30	60	90	15	30	45	60	75
Total phenols (mg/kg)	I	601.4 ± 7.1^{a}	649.4 ± 7.0^{b}	659.2 ± 7.1^{b}	674.6 ± 5.6^{a}	673.9 ± 7.1^{a}	724.2 ± 5.6^{b}	701.7 ± 6.3^{a}	698.7 ± 9.9^{a}
	II	427.2 ± 9.5^{a}	408.8 ± 2.2^{a}	418.1 ± 9.7^{a}	419.0 ± 2.7^{b}	414.3 ± 6.2^{b}	481.2 ± 8.5^{c}	411.6 ± 8.5^{b}	342.5 ± 5.7^{a}
o-Diphenols (mg/kg)	I	324.5 ± 6.3^{a}	326.5 ± 3.5^{a}	308.2 ± 5.1^{a}	440.0 ± 7.0^{c}	429.2 ± 9.8^{b}	443.8 ± 2.1^{c}	426.9 ± 9.8^{c}	357.7 ± 5.6^{a}
	II	212.6 ± 9.0^{a}	183.7 ± 3.1^{a}	181.2 ± 7.5^{a}	239.5 ± 8.7^{c}	224.6 ± 9.1^{c}	225.4 ± 8.5^{c}	192.5 ± 8.7^{b}	84.6 ± 8.5^{a}
α-Tocopherol (mg/kg)	I	207 ± 2^{b}	219 ± 1^{c}	200 ± 3^{a}	226 ± 3^{b}	223 ± 2^{b}	194 ± 3^{a}	220 ± 1^{b}	222 ± 2^{b}
	II	206 ± 2^{b}	188 ± 3^{a}	192 ± 2^{a}	246 ± 5^{b}	192 ± 3^{a}	201 ± 2^{a}	202 ± 4^{a}	193 ± 3^{a}
β-Carotene (mg/kg)		1.06 ± 0.11^{b}	0.87 ± 0.10^{a}	1.34 ± 0.13^{b}	–	–	–	–	–
Oxidative stability (h)	I	20.6 ± 0.1^{a}	21.8 ± 0.2^{b}	20.1 ± 0.2^{c}	17.5 ± 0.7^{a}	25.2 ± 0.3^{d}	23.8 ± 0.3^{c}	27.3 ± 0.4^{e}	20.9 ± 0.1^{b}
	II	20.5 ± 0.7^{b}	$18.3 \pm 0.9^{a,b}$	16.7 ± 0.4^{a}	23.9 ± 0.1^{e}	22.9 ± 0.5^{d}	19.4 ± 0.4^{c}	18.2 ± 0.3^{b}	12.4 ± 0.5^{a}
DPPH test (meq Trolox/kg)	I	1435 ± 49^{a}	1520 ± 28^{a}	1409 ± 57^{a}	2268 ± 16^{c}	2359 ± 12^{d}	2124 ± 33^{b}	2443 ± 13^{e}	1832 ± 45^{a}
	II	1220 ± 28^{c}	730 ± 42^{a}	958 ± 2^{b}	1849 ± 15^{e}	1511 ± 16^{d}	744 ± 8^{b}	1030 ± 48^{c}	513 ± 19^{a}
K_{225}	I	0.55 ± 0.01^{c}	0.52 ± 0.03^{b}	0.48 ± 0.01^{a}	0.59 ± 0.01^{c}	0.59 ± 0.02^{c}	0.57 ± 0.02^{b}	0.61 ± 0.01^{d}	0.51 ± 0.02^{a}
	II	0.48 ± 0.02^{b}	0.47 ± 0.01^{b}	0.41 ± 0.01^{a}	0.58 ± 0.01^{c}	0.57 ± 0.01^{c}	0.52 ± 0.02^{b}	0.51 ± 0.01^{b}	0.42 ± 0.02^{a}

Malaxation temperature constant: 28 °C

Different letters within a component (a–d) indicate significant differences ($p < 0.05$)

90 min) according to the behaviour of the *o*-diphenols. This observed effect could be very important from a sensory point of view in biophenol-rich olive oil varieties like Cornicabra, in which bitterness can sometimes be so excessive as to cause consumer rejection of the product. Appropriate control of the technological variables examined here could therefore produce a desirable reduction of the intensity of this attribute and hence improve consumer preference.

In selecting optimal kneading conditions in the Cornicabra variety, a suitable compromise needs to be reached between the oil yield and the overall quality of the virgin olive oil, the latter mainly related to its minor components of sensory and antioxidant value. Therefore, the results of this experiment suggest that the best malaxation conditions may be a temperature below 28 °C and a time longer than 60 min, since the phenolic content of the virgin olive oil obtained would enhance the sensory properties of this variety by reducing bitterness; moreover the expected decrease in the oxidative stability would not affect the shelf-life of Cornicabra virgin olive oil, as this is a highly stable and biophenol rich variety.

Acknowledgments This research project was supported by Project PBI05-047 of the Junta de Comunidades de Castilla-La Mancha.

References

1. Luchetti F (2002) Eur J Lipid Sci Technol 104:559–563
2. Caponio F, Gomes T, Summo C, Pasqualone A (2003) Eur J Lipid Sci Technol 105:201–206
3. Di Giovachino L, Sestili S, Di Vincenzo D (2002) Eur J Lipid Sci Technol 104:587–601
4. Servili M, Piacquadio P, De Stefano G, Taticchi A, Sciancalepore V (2002) Eur J Lipid Sci Technol 104:483–489
5. Almirante R, Cini E, Montel GL, Pasqualone A (2001) Grasas y Aceites 52:198–201
6. Ranalli A, Contento S, Schiavone C, Simone N (2001) Eur J Lipid Sci Technol 103:228–238
7. Jiménez-Márquez A, Hermoso-Fernández M, Uceda-Ojeda M (1995) Grasas y Aceites 46:299–303
8. Servili M, Selvaggini R, Taticchi A, Esposto S, Montedoro G (2003) J Am Oil Chem Soc 80:685–695
9. Angerosa F, Mostallino R, Basti C, Vito R (2001) Food Chem 72:19–28
10. Di Giovachino L, Costantini N, Ferrante ML, Serraiocco A (2002) Grasas y Aceites 53:179–186
11. Ranalli A, Pollastri L, Contento S, Lannucci E, Lucera L (2003) Eur J Lipid Sci Technol 105:57–67
12. Lercker G, Frega N, Bocci F, Mozzori M (1999) Grasas y Aceites 50:26–29
13. IOOC (1984) Document no 6. International Olive Oil Council, Madrid
14. UNE (1973) Spanish Standard UNE 55032
15. European Union Commission (1991) Off J Eur Commun 2568:91
16. Mateos R, Espartero JL, Trujillo M, Ríos JJ, León-Camacho M, Alcudia F, Cert A (2001) J Agric Food Chem 49:2185–2192
17. AOCS (1998) Official methods and recommended practises of the American Oil Chemist Society, 5th Champaign, Illinois
18. Gimeno E, Calero E, Castellote AI, Lamuela-Raventós RM, de la Torre MC, López-Sabate MC (2000) J Chrom 881:255–259
19. Williams B (1995) Lebens–Wiss U–Technology 28:25–30
20. Laubi MW, Bruttel PA (1986) J Am Oil Chem Soc 63:792–795
21. Gutiérrez-Rosales F, Perdiguero S, Gutiérrez R, Olías JM (1992) J Am Oil Chem Soc 69:394–395
22. European Union Commission (2007) Off J Eur Commun 702/2007
23. Kalua CM, Bedgood DR Jr, Bishop AG, Prenzler PD (2006) J Agric Food Chem 54:7641–7651
24. Martel J, Alba-Mendoza J (1981) Grasas y Aceites 8:112–120
25. Rodis PS, Karathanos VT, Mantzavinou A (2002) J Agric Food Chem 50:596–601
26. Di Giovacchino L (1991) Riv Ital Sos Grasse 68:314–420
27. Servili M, Baldioli G, Montedoro G (1994) Acta Hort 356:331–336