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Are virgin olive oils obtained below 27 °C better than those produced at higher temperatures?

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ABSTRACT

Within the European Union, indications of 'first cold pressing' and 'cold extraction' can only be used for virgin olive oil (VOO) obtained below 27 °C from mechanical processing. Three different malaxing temperatures (25, 35 and 45 °C) are here evaluated for the quality of the VOO obtained in a continuous industrial plant. The oils were stored at room temperature in the dark for 12 months. Initially, oil obtained from a blend of Frantoio/Leccino cultivars (F/L) had higher acidity and peroxide levels and lower phenolic content than a Coratina cultivar (Cor). The oxidative stability of the oils positively correlated with malaxation temperature (F/L, $R^2 = 0.818$; Cor, $R^2 = 0.987$) as the phenolic content was directly proportional to the temperature (F/L, $R^2 = 0.887$; Cor, $R^2 = 0.992$). Only oils obtained at 45 °C were rejected because of 'heated or burnt' off-flavour. Decarboxymethylation of secoiridoids and further hydrolysis of phenolic esters occurred during storage. The oxidation products of derivatives of tyrosol and hydroxytyrosol were detected after nine months in both the F/L and Cor samples. Thus, VOO obtained at a processing temperature lower than 27 °C does not show higher chemical and sensory qualities than VOO obtained at 35 °C.

1. Introduction

In 2002, European Commission (EC) Regulation No. 1019/2002 introduced new marketing standards for olive oil. According to Art. 5: (a) the indication 'first cold pressing' can only be used for VOO or extra-VOO obtained at temperatures below 27 °C from the first mechanical pressing of olive paste by a traditional extraction system using hydraulic presses; (b) the indication 'cold extraction' can only be used for VOO or extra-VOO obtained at temperatures below 27 °C by percolation or centrifugation of the olive paste.

This setting of a threshold of 27 °C for a specific labelling rule in the VOO market has generated much criticism, as the climatic conditions of many oil-producing regions prevent the maintenance of this temperature during the processing of olives. Furthermore, this rule appeared to be based on arbitrary commercial discrimination, and not on experimental evaluation of oil quality.

Olive oil quality is related to the chemical composition of the oil, and its oxidative stability and sensory characteristics. These parameters are affected by pedoclimatic conditions (Bonoli, Bendini, Cerretani, Lercker, & Gallina Toschi, 2004; Morellò, Romero, & Motilva, 2006; Nergiz & Engez, 2000), agronomic practices (Romero, Tovar, Girona, & Motilva, 2002; Servili et al., 2007), olive *cultivar* (Beltràn, Aguilera, Del Rio, Sanchez, & Martinez, 2005; Olivares-Lopez et al., 2007), degree of maturity of the olives (Rotondi et al., 2004; Salvador, Aranda, & Fregapane, 2001), and last but not least, the parameters of the processing technology in the olive mills (Cerretani, Bendini, Rotondi, Lercker, & Gallina Toschi, 2005; Di Giovacchino, Sestili, & Di Vincenzo, 2002). A fundamental phase of the extraction process for olive oil is the malaxation of the olive paste. The olive paste that is obtained from the olive crushing has to be malaxed to improve the successive separation steps and to increase the yield of the oil extraction. The oil droplets inside the oil-bearing cells are partly located in the vacuole (about 76%), where the oil is in a free form, and partly located in the cytoplasm (about 24%), where the oil is dispersed and bound to colloids through the lipoprotein membranes (Ranalli, Malfatti, & Cabras, 2001). The slow stirring of the paste brings the colloid-bound oil droplets into contact with each other. Through this interaction, the oil combines into large droplets, the radius of which overcomes the force that binds them to the colloid system, according to Stoke's law. Finally, the oil droplets are released due to the phase inversion of the emulsion. This process is known as *coalescence*, and it results in the formation of free oil (droplets with diameters $> 30\ \mu m$) (Martinez Monero, Gomez Herrera, & Janer del Valle, 1957; Rangel, Platt, & Thomson, 1997), which is then easily extracted by physical and mechanical means.

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Table 1
The oil quality parameters.

	25 °C					35 °C					45 °C				
Time (months)	T_0	T_3	T_6	T_9	T_{12}	T_0	T_3	T_6	T_9	T_{12}	T_0	T_3	T_6	T_9	T_{12}
F/L															
Free acidity[a]	0.30	0.30	0.31	0.37	0.34	0.30	0.30	0.33	0.37	0.37	0.40	0.40	0.34	0.41	0.39
Total phenols[b]	84.7	92.9	109	122	99.4	133	143	179	157	128	143	145	170	160	140
Induction period (h)	21.4	19.5	18.1	19.5	19.6	28.9	24.8	24.3	23.1	24.8	29.4	24.2	24.5	22.6	21.4
Cor															
Free acidity[a]	0.20	0.20	0.26	0.25	0.22	0.20	0.20	0,21	0.22	0.22	0.30	0.30	0.22	0.28	0.25
Total phenols[b]	172	235	259	234	201	201	274	270	327	270	241	359	326	351	384
Induction period (h)	43.70	40.7	40.0	37.3	39.1	47.8	42.4	41.6	41.7	41.2	53.9	51.2	48.9	48.6	50.5

F/L, Frantoio/Leccino; Cor, Coratina. 25 °C, 35 °C and 45 °C: different malaxation temperatures; T_0, freshly obtained oils; T_3, T_6, T_9, and T_{12} months of storage.
[a] g Oleic acid in 100 g of oil.
[b] mg of Gallic acid kg^{-1} of oil.

The technological parameters during this step, with particular reference to time and temperature, have very important roles in determining the yield of oil and the chemical and sensory characteristics of the final product (Angerosa, Mostellino, Basti, & Vito, 2001; Koutsaftakis, Kotsifaki, & Stefanoudaki, 1999; Ranalli, Contento, Schiavone, & Simone, 2001). It has been shown that prolonging the malaxation time up to 90 min at a temperature of up to 35 °C provides increased oil yield (Di Giovacchino, 1991). Thus, the processing temperature during malaxation is often kept at 35–40 °C in the olive mills for economic reasons. However, during malaxation, chemical and enzymatic reactions take place that can markedly modify the composition of the oil. The rate and extent of these reactions are greatly affected by the parameters adopted. Increasing the temperature during the olive paste malaxing process increases the enzymatic activity of the oxidoreductases that are naturally present in olive pulp, such as polyphenoloxidase (PPO), lipoxygenase (LOX) and peroxidase (POD). According to Servili, Baldioli, Selvaggini, Mariotti, and Montedoro (1998), PPO is almost completely inactivated during the crushing of olives, and the only active enzymes during the oily paste malaxation are LOX and POD (Servili et al., 1998). LOX catalyses the formation of hydroperoxides and can also promote indirect oxidation of secoiridoids. Another enzyme that is active here is β-glucosidase, which has a role in the production of phenol-aglycones (secoiridoids) through hydrolysis of oleuropein and demethyloleuropein glycosides (Ranalli, Pollatri, Contento, Iannucci, & Lucera, 2003; Servili, Baldioli, Mariotti, & Montedoro, 1999; Servili, Selvaggini, Taticchi, Esposto, & Montedoro, 2003).

In the present study, we have investigated the effects of three different malaxation temperatures (25 °C, 35 °C and 45 °C) on the quality parameters, stability and phenolic profile of VOO obtained from two blends of olive fruits (F/L and Cor), both as fresh oil and following a storage period of 12 months.

2. Materials and methods

2.1. Olive oil extraction and storage

Two olive batches were processed. The first was a mixture of Frantoio and Leccino cultivars (F/L) (about 1400 kg; 1:1, w/w) that were from olive trees grown in the Marches Region in Italy. The second batch consisted of 100% Coratina (Cor) olives (about 1400 kg) that were from olive trees grown in the Puglia Region in Italy. Three subsamples of about 465 kg of olives were obtained from each batch (one for each of the three different malaxing temperatures).

The olives were processed in a two-way continuous industrial plant (Pieralisi Group, Jesi, Italy). They were defoliated and washed prior to crushing, and then processed using a system with a mobile hammer crusher and a malaxer (Genius P4 model, Pieralisi group, Jesi, Italy); the olive paste malaxation lasted 45 min. Each of three subsamples (F/L and Cor) was subjected to a different malaxation temperature: 25 °C, 35 °C and 45 °C. The oil was then extracted using a horizontal centrifuge (decanter) operating at $2410 \times g$ (Maior I "special" model, Pieralisi group, Jesi, Italy). Separation of the resulting oily must into oil and water was achieved in a vertical automated discharge centrifuge. The oil samples were poured into 30 bottles (750 mL each) of green glass which were sealed with a screw cap; the headspace in the bottles was approximately 10 mL. Six bottles were immediately opened for the fresh analysis (at T_0) and the remaining 24 bottles were stored in the dark at room temperature for the following storage intervals: three months (T_3), six months (T_6), nine months (T_9) and 12 months (T_{12}). Six bottles were opened for each analysis.

2.2. Determination of legal quality parameters

Free acidity (% oleic acid per 100 g olive oil), peroxide level (meq O_2 kg^{-1} oil) and UV determinations (ΔK) were carried out for each oil sample (at T_0, T_3, T_6, T_9 and T_{12}) according to the European Community (EC, 1991) and International Olive Oil Council (IOOC, 2003) standard methods. The parameters K_{232} and K_{270} are the oil absorbance at 232 and 270 nm, respectively, whereas ΔK was calculated from the absorbances at 262, 268 and 274 nm. Spectrophotometric determinations, K_{232}, K_{270}, ΔK and total phenols were carried out using a UV-Vis-Nir *Cary* 5000 Varian spectrophotometer (Leinì, Italy).

Sensory evaluation was carried out by eight judges who were fully trained in the evaluation of VOO according to the official methods of the IOOC (1996).

2.3. Fatty acid methyl ester analysis by gas chromatography

The fatty acid methyl esters (FAMEs) of the oil samples were obtained by alkaline treatment with 1 M KOH in methanol

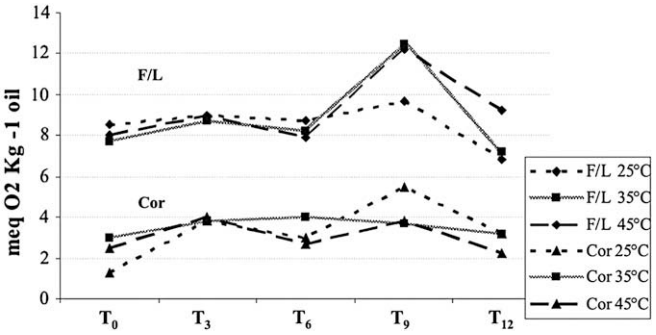

Fig. 1. Trends for the peroxide levels of VOO over the 12 months of storage.

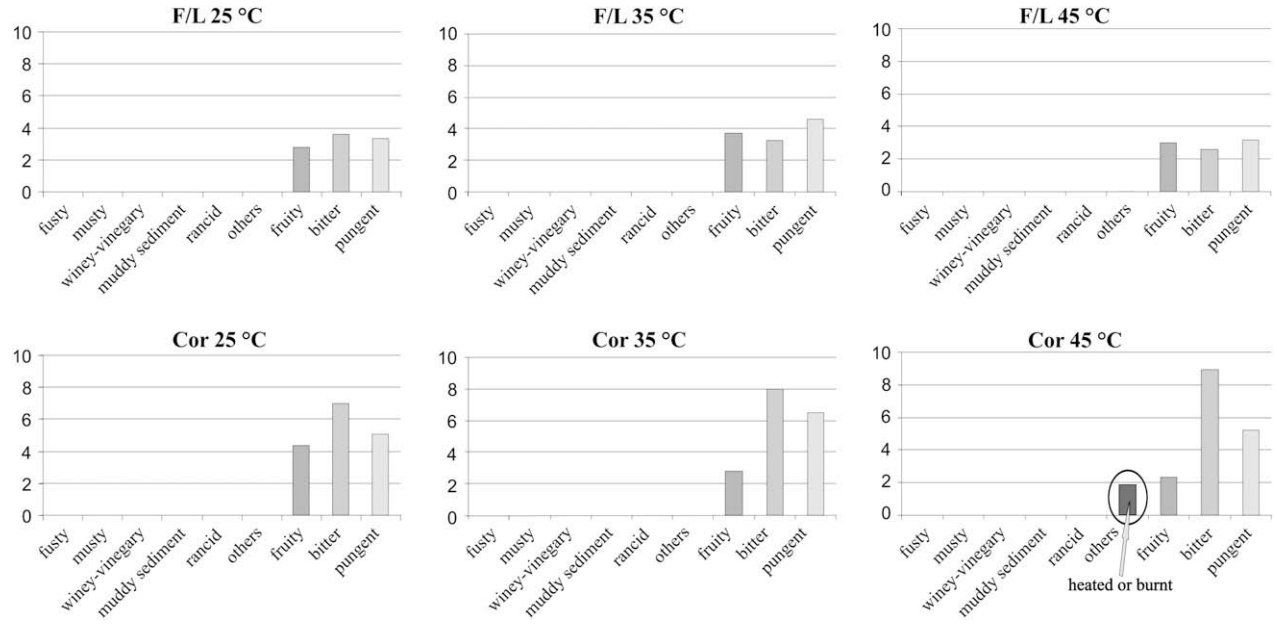

Fig. 2. Sensory scores of the fresh VOO samples (at T_0).

(Christie, 1998). These were then injected into a HRGC Mega 2 series Model MFC 800 gas chromatography system (Fisons Instruments, Milan, Italy). This was equipped with a flame ionization detector and a fused silica capillary column (SP 2330: 60 m length × 0.25 mm i.d. and 0.2 μm film thickness; Supelco, St. Louis, MO, USA). The carrier gas was helium (2 mL min^{-1}), and the splitting ratio was 1:80. The injector and detector temperatures were 250 °C, with a temperature programme that started from 150 °C and raised the temperature to 220 °C at a rate of 3 °C min^{-1}, where it was held for 30 min. The FAMEs were identified by their retention times in comparison with an authentic standard mix containing the FAMEs from C10 to C22 (Sigma-Aldrich Co. Ltd., UK).

2.4. Determination of the oxidative stability

The oxidative stability of the oils was determined with a Rancimat apparatus (Metrohm model 679, Herisau, Switzerland), which measured the induction period of a 5-g oil sample heated to 110 °C under an air flow of 20 L h^{-1}. The induction period was determined by drawing the two tangents of the time–conductivity curve and projecting the intersection onto the time-axis. The induction period was expressed in h.

2.5. Extraction of the phenolic fraction

Aliquots of oil (5 g) were added to 10 mL of a methanol/water solution (80:20, v/v) in a 50-mL centrifuge tube, according to Montedoro et al. (1992). The mixture was blended (Ultraturrax, IKA, Staufen, Germany) for 5 min and then centrifuged for 5 min at 2500 × *g*. The hydroalcoholic extract was collected and the oil phase was re-extracted with 2 × 10 mL methanol/water solution. Finally, the hydroalcoholic fractions were combined and washed with *n*-hexane to remove residual oil, and then concentrated and dried under vacuum at 35 °C. For spectrophotometric analysis, the dry extracts were resuspended in 1 mL methanol. For HPLC analysis, 100 μL of an internal standard of 3,4-dihydroxyphenylacetic acid solution (10 μg mL^{-1}) was added to 5 g oil. After the extraction procedure, the dry extracts were resuspended in 1 mL methanol and the solutions were filtered through 0.2 μm regenerated cellulose filters (Schleicher & Schuell, Dassel, Germany).

2.6. Spectrophotometric determination of total phenols

The total phenolic content of the hydroalcoholic extract was determined according to the Singleton spectrophotometric method at 765 nm (Singleton & Rossi, 1965). The results were expressed as gallic acid equivalents (mg kg^{-1} oil) based on the calibration curve ($R^2 = 0.996$). Folin–Ciocalteu reagent and gallic acid were obtained from Merck & Co. Inc. (Darmstadt, Germany).

2.7. High performance liquid chromatography of phenols

The qualitative and quantitative characterization of phenolic compounds was obtained by HPLC with a diode array detector (DAD). A Chrompack (Middelburg, The Netherlands) 25 cm × 4.6 mm i.d. column packed with Chromspher C18 (5-μm particle size), a Varian 9010 ternary pump delivery system (Walnut Creek, CA, USA) and a GasTorr 154 degasser (Tokyo, Japan) were used. The samples were injected into a 20-μL loop, with a mobile-phase flow rate of 700 μL min^{-1}. Gradient elution was carried with a solvent system of water/acetic acid (98:2 v/v) as mobile phase A and methanol as mobile phase B; the total run time was 75 min (Boselli, Di Lecce, Minardi, Pacetti, & Frega, 2007). All the solvents were filtered through 0.45 μm nylon filter disks (Sartorius AG, Goettingen, Germany) prior to use. The detector was a Varian Prostar PDA 330 (Walnut Creek, CA, USA). The DAD monitored phenolic acids, phenyl ethyl alcohols and secoiridoids at 280 nm, and flavones at

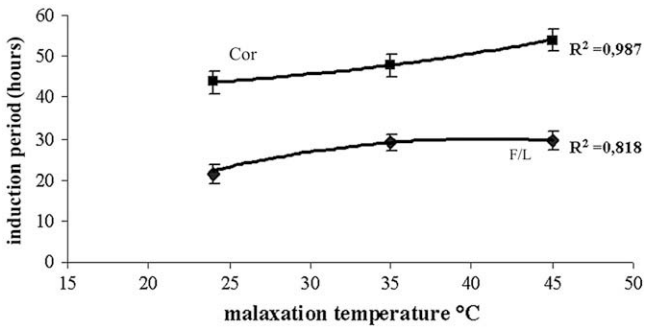

Fig. 3. Correlation of malaxation temperatures with oxidative stability of the VOO.

Table 2
Fatty acid methyl esters contents of the VOO after 12 months of storage (T_{12}).

	Fatty acid methyl ester contents (%)					
	F/L			Cor		
	25 °C	35 °C	45 °C	25 °C	35 °C	45 °C
C 16:0	11.5	11.6	11.5	10.2	10.1	10.1
C 16:1	0.83	0.68	0.63	0.23	0.24	0.26
C 18:0	1.61	1.59	1.60	1.97	1.98	1.99
C 18:1	78.7	78.7	78.7	80.4	80.6	80.5
C 18:2	5.99	6.05	6.07	5.67	5.64	5.62
C 20:0	0.32	0.30	0.34	0.37	0.38	0.36
C 18:3	0.66	0.69	0.71	0.71	0.70	0.74
C 20:1	0.35	0.36	0.39	0.43	0.41	0.41
C18:1/C18:2	13.2	13.0	13.0	14.2	14.3	14.3
Insaturation index[a]	153	154	154	152	152	152

F/L, Frantoio/Leccino; Cor, Coratina.
C *n*:*m*. *n* = number of carbon atoms; *m* = number of double bonds.
[a] Calculated as ∑[% monounsaturated + (diunsaturated × 10) + (triunsaturated × 20)]/100.

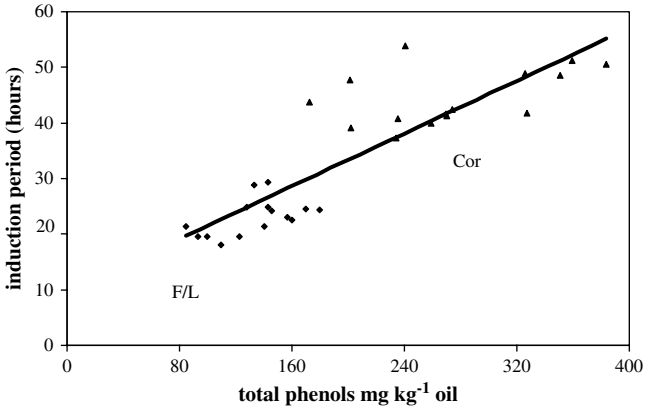

Fig. 4. Correlation of total phenols with oxidative stability in the VOO.

350 nm. The data were acquired using Varian Star 6.3 software (Walnut Creek, CA, USA). Two phenolic alcohols and one phenolic acid were quantified using a calibration curve obtained with *p-hydroxyphenylethanol* ($R^2 = 0.998$); vanillic acid and vanillin were quantified using their respective standards ($R^2 = 0.996$ and 0.998, respectively). Six secoiridoids were quantified with 3,4-dihydroxyphenylacetic acid ($R^2 = 0.999$), and three flavones were quantified with quercetin ($R^2 = 0.998$). The standards for HPLC quantification were purchased from Extrasynthèse (Genay, France), Sigma (St. Louis, MO, USA) and Fluka (Buchs, Switzerland). For structural elucidation, the HPLC system was coupled online to an LCQ ion-trap mass spectrometer (Thermoquest, San José, CA, USA) equipped with an electrospray ionization source suitable for tandem mass spectrometry (MS-MS). The LC effluent was split and 0.1 μL min^{-1} entered the mass spectrometer through a steel ionization needle set at 4 kV and a heated capillary set at 200 °C. The sheath gas flow was approximately 70 arbitrary units. Tandem mass experiments were performed with a relative collision energy of 30–40%. All of the data were acquired with Excalibur software Version 1.2 (Thermoquest).

Mean concentrations were calculated from three replications of each sample.

2.8. Statistical analysis

The chemical data were analysed using Statistica 6.0 (Statsoft Inc., Tulsa OK, USA) statistical software. The significances of differences at a 5% level between the means were determined by one-way ANOVA, using Tukey's test.

3. Results and discussion

3.1. Free acidities, peroxide levels and UV determinations

The trends in the free acidities and peroxide levels in the two different oil batches from T_0 over the 12-month storage period are shown in Table 1. The time evolution of the peroxide levels is shown in Fig. 1. These parameters were minimally affected by malaxation temperature: the oils obtained with the F/L blend at T_0 showed a free acidity ranging from 0.3 to 0.4 mg oleic acid per 100 mg sample. Free acidity did not increase during the 12 months of storage and remained under the legal limit (0.8% oleic acid). The VOO obtained with the Cor cv. had significantly lower free acidity ($P < 0.0001$) than the F/L blend, ranging from 0.2% to 0.3%. Also for Cor the free acidity did not increase during storage. Increasing the malaxation temperature to 45 °C caused only a small increase in the free acidity (0.1%) at T_0 of both of the olive oil batches.

Very low peroxide levels were detected in all of the freshly obtained samples (at T_0) under all of the malaxation temperatures; these were stable over the 12 months of storage (Fig. 1) and did not exceed the legal threshold (20 meq O_2 kg^{-1} oil). However, the peroxide levels of the F/L oils ranged from 7.7 to 8.5 meq O_2 kg^{-1}, whereas those for the Cor oils had significant lower peroxide content ($P < 0.0001$), which ranged from 1.3 to 3.0 meq O_2 kg^{-1}. The differences in the peroxide levels between the two oil types might

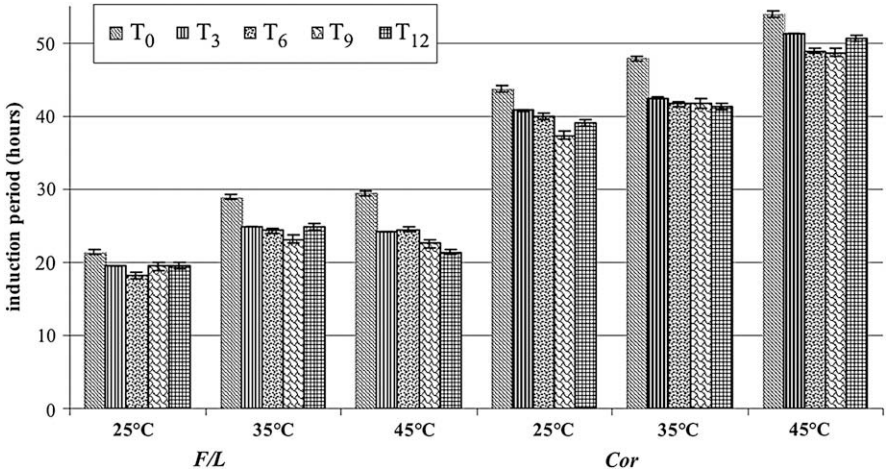

Fig. 5. Induction time of the VOO samples during the 12 months of storage.

Table 3
Quantitative data for different malaxation temperatures over the five storage periods.

	25 °C					35 °C					45 °C				
	T_0	T_3	T_6	T_9	T_{12}	T_0	T_3	T_6	T_9	T_{12}	T_0	T_3	T_6	T_9	T_{12}
F/L															
3.4-DHPEA[a]	–	5.81b	18.5g,h	11.7c,d	13.9d,e	2.12a	7.81b	16.9f,g	11.1c	14.8e,f	2.00a	7.73b	19.7h	15.2e,f	22.3i
p-HPEA[a]	1.85a	6.40c,d	14.7g	9.37e	11.9f	3.61b	5.92c,d	8.89e	5.40c	5.72c,d	3.11a,b	6.22c,d	9.02e	7.14d	12.9f
Vanillic acid[b]	0.25b,c	0.27c,d	0.33d	0.15a	0.20a,b	0.48f	0.56g	0.57g	0.30c,d	0.27c,d	0.40e	0.45e,f	0.45e,f	0.27b,c,d	0.24b,c
Vanillin[c]	0.67c	0.64c	0.85c,d,e	0.37a,b	0.36a	1.16f,g	1.35g	1.36g	0.70c,d	0.61b,c	1.66h	1.93i	1.87h,i	1.03e,f	0.93d,e,f
3.4-DHPEA-Ac[a]	1.51b	1.31b	1.17 b	0.30a	0.43a	3.89c	4.60d	4.35c,d	1.85b	2.09a	4.97d	4.76d	4.94d	2.08b	2.18b
3.4-DHPEA-EDA[d]	16.0c	14.5c	13.4b,c	3.1a	1.69a	40.6d	46.2d,e	44.8d,e	14.7f	8.89a,b,c	49.9e	42.8d,e	43.0d,e	14.2c	6.67a,b
3.4-DHPEA-EDA-Ox[d]	–	–	–	0.46a	0.35a	–	–	–	1.68a	1.42a	–	–	–	1.41a	7.57b
p-HPEA-EDA[d]	73.5c,d	72.7c,d	68.5c	7.34a,b	4.25a	94.0f	110g	81.7e	12.9b	8.93a,b	95.9g	110f	77.1d,e	13.3b	7.30a,b
DLA[4] + *p*-HPEA-EDA-Ox[d]	–	–	–	27.1a	27.5a	–	–	–	37.5c,d	35.3b	–	–	–	42.2d	39.7c,d
3.4-DHPEA-EA[d]	15.1c,d	14.5c	10.1b	3.81a	4.05a	22.7g,h	24.3g,h	18.9e,f	5.62a	7.10a,b	25.5h	21.9f,g	18.2d,e	6.30b	5.09a
LA[d]	–	–	–	4.12a,b	3.78a	–	–	–	4.60b,c	4.14a,b	–	–	–	5.60d	4.98c,d
Luteolin[e]	1.86b,c	1.88b,c	1.94c	–	–	1.91c	1.94c	1.77b,c	–	–	1.72a,b,c	1.66a,b	1.51a	–	–
Apigenin[e]	0.77f	–	–	0.37b,c,d	0.44d,e	0.39c,d	–	–	0.27a	0.32a,b	0.49e	–	–	0.28a	0.32a,b,c
Methoxyluteolin[e]	–	–	–	0.41b	0.43b	–	–	–	0.25a	0.24a	–	–	–	0.29a	0.28a
Sum of phenols	111	118	129	68.6	69.4	171	202	175	96.8	89.9	186	198	171	109	111
Cor															
3.4-DHPEA[a]	14.9a	17.4a,b,c	28.4b,c,d	31.8d,e	29.6c,d	17.8a,b,c	17.1a,b	58.0g	52.2f,g	47.5f,g	17.0a,b,c	9.34a	42.2e,f	49.0f	48.8f,g
p-HPEA[a]	18.7c,d,e	7.84a	10.4a,b	14.1b,c	11.4a,b	27.1f	11.5a,b	35.3g	24.1e,f	23.5d,e,f	17.3b,c	6.88a	19.2c,d,e	21.0c,d,e	17.8c,d
Vanillic acid[b]	0.54b,c,d,e	0.60d,e	0.57c,d,e	0.31a,b	0.26a	0.54b,c,d,e	0.51b,c,d,e	0.59d,e	0.35a,b,c	0.32a,b	0.62e	0.62e	0.51b,c,d,e	0.40a,b,c,d,e	0.47a
Vanillin[c]	0.21a,b,c,d	0.25b,c,d,e	0.36d,e	0.06a	0.07a	0.32c,d,e	0.27b,c,d,e	0.34c,d,e	0.11a,b	0.12a,b	0.61g	0.55f,g	0.41e,f	0.22a,b,c	0.26b,c,d,e
3.4-DHPEA-Ac[a]	–	–	–	0.14a	0.17a	–	–	–	0.38b	0.48c	–	–	–	0.37b	0.54c
3.4-DHPEA-EDA[d]	39.5a,b,c,d	62.0c,d,e,f	57.4b,c,d,e	19.6a,b	14.9a	67.5d,e,f	89.0e,f,g	101f,g,h	41.7a,b,c,d	22.7a,b,c	118h	186i	113g,h	59.7a,b,c,d,e	55.1c,d,e,f
3.4-DHPEA-EDA-Ox[d]	–	–	–	4.44b	2.72a	–	–	–	7.31c	3.02a,b	–	–	–	16.9e	13.4d
DOA[d]	–	72.5e	53.1d	8.15a	4.76a	–	50.1c,d	47.8c,d	6.98a	4.32a	–	27.0b	33.5b,c	5.67a	6.13a
p-HPEA-EDA[d]	101b,c	114b,c	86.1b	22.8a	17.8a	132c,d	122c,d	118b,c	39.3a	24.0a	152d,e	176e	103b,c	50.8a	46.6a
DLA[d]	–	55.9c	37.7b	–	–	–	36.7b	27.2b	–	–	–	27.7b	10.1a	–	–
DLA[d] + *p*-HPEA-EDA-Ox[d]	–	–	–	31.9a,b	30.5a	–	–	–	38.2b,c	31.1a,b	–	–	–	36.3a,b	37.3c
3.4-DHPEA-EA[d]	95.9f	48.1a,b,c,d	45.6a,b,c,d	37.4a,b	35.5a	87.3f	60.9d	58.2c,d	49.9a,b,c,d	38.1a,b,c	62.4d,e	83.5e,f	55.7b,c,d	53.7a,b,c,d	55.0b,c,d
LA[d]	62.3e	22.1b,c	27.8c,d	8.11a	8.11a	56.5e	26.7c,d	31.5c,d	9.42b	7.49a	35.5d	34.3d	26.8c,d	10.4a	13.8a,b
Luteolin[e]	1.40a,b,c	2.34d	1.81b,c,d	1.43b,c,d	1.22a	1.14a	1.66a	1.88c,d	1.36a,b,c	1.17a	1.19a,b	1.63a,b,c	1.16a	1.18a	1.25a,b
Apigenin[e]	0.65a	0.91a	0.75a	0.54a	0.50a	0.53a	0.64a	0.73a	0.52a	0.48a	0.45a	0.57a	0.49a	0.42a	0.46a
Methoxyluteolin[e]	–	–	–	0.18b	0.12a	–	–	–	0.18b	0.12a	–	–	–	0.13a	0.11a
Sum of phenols	335	404	345	181	158	390	417	480	262	209	405	555	407	279	308

F/L, Frantoio/Leccino; Cor, Coratina.
25 °C, 35 °C and 45 °C, different malaxation temperatures; T_0, T_3, T_6, T_9 and T_{12} analysis periods.
3,4-DHPEA: 3,4-dihydroxyphenylethanol; *p*-HPEA: *p*-hydroxyphenylethanol; 3,4-DHPEA-Ac: 3,4-dihydroxyphenylethanol-acetate; 3,4-DHPEA-EDA: dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA; 3,4-DHPEA-EDA oxidized; DOA: dialdehydic form of oleuropein aglycon; *p*-HPEA-EDA: dialdehydic form of decarboxymethyl elenolic acid linked to *p*-HPEA; DLA: dialdehydic form of ligstroside aglycon; *p*-HPEA-EDA-oxidized; 3,4-DHPEA-EA: oleuropein aglycon; LA: ligstroside aglycon.
Different letters in the same row indicate significantly different values ($P < 0.05$).
[a] mg *p*-Hydroxyphenylethanol kg^{-1} of oil.
[b] mg vanillic acid kg^{-1} of oil.
[c] mg vanillin kg^{-1} of oil.
[d] mg 3.4-dihydroxyphenylacetic acid kg^{-1} of oil.
[e] mg quercetin kg^{-1} of oil.

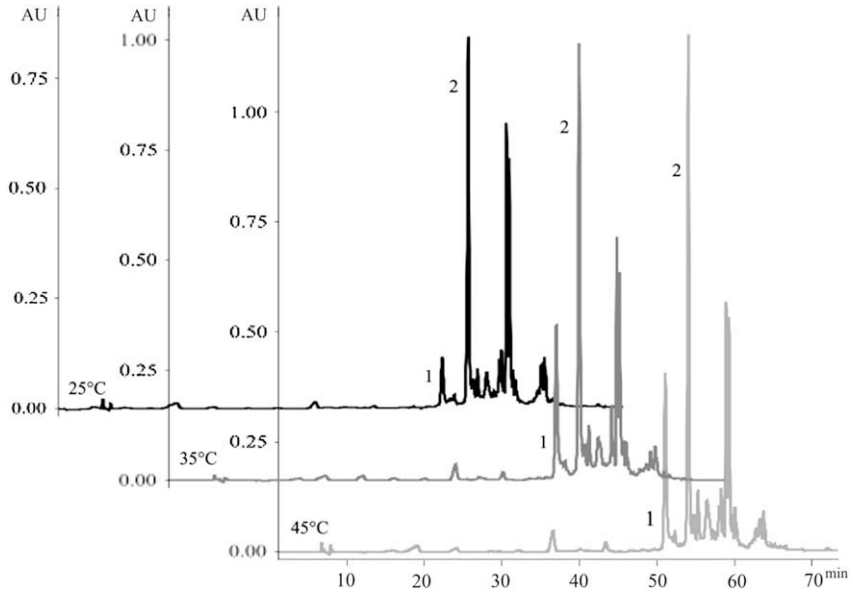

Fig. 6. HPLC–DAD–MS/MS phenolic profiles of the Cor oils (T_3) obtained at the three different malaxation temperatures. 1, 3,4-DHPEA-EDA; 2, *p*-HPEA-EDA.

arise from several factors, such as the degree of maturity of the olives, their delivery and storage conditions, and the extent of pest attacks.

The UV determinations did not show significant differences between the samples. All of the values for the fresh oils and after storage for 12 months were lower than the legal limits fixed for extra-VOOs ($K_{232} \leq 2.4$, $K_{270} \leq 0.2$ and $\Delta K \leq 0.01$).

3.2. Sensory evaluation

The sensory profiles of the F/L and Cor oils obtained using malaxation temperatures of both 25 °C and 35 °C were very good, unlike those obtained at 45 °C. These lower malaxation temperatures (i.e. 25 °C and 35 °C) avoided any negative attributes according to both EC Reg. n. 796/2002 and the new EC Reg. n. 640/2008, which will apply from October 2008. The oils obtained at 45 °C showed the negative attribute of 'heated/burnt' (EC Reg. 796/2002), which was more evident (Fig. 2) in the Cor oils (median 2.0) than in the F/L oils (3 of 8 judges perceived this defect, thus the median was 0.0).

The fruity score range of the F/L oils was from 2.9 to 3.7, while for the Cor oils it ranged from 2.4 to 4.3. Although in the F/L oils the fruity score was not affected by the malaxation temperature increase, in the Cor oils there was a clear decrease in this score for increased malaxation temperature.

The F/L oils showed a (positive) bitter attribute (always lower than the Cor oils) which decreased slightly with increased malaxation temperature. The Cor oils showed an opposite trend, indicating a correlation of their bitter attribute with the phenolic content.

The (positive) pungent attribute showed a bell-shaped trend with malaxation temperature for both oils, with the highest scores obtained at 35 °C.

3.3. Total phenolic content, oxidative stability and fatty acid profile

VOO is known to contain a higher amount of phenolic compounds with respect to plant oils, which are solvent-extracted and then refined. These compounds contribute to the complex global flavour (Kiritsakis, 1998), provide antioxidant effects (Del Carlo et al., 2004; Franconi et al., 2006; Lavelli, 2002), and are largely responsible for the shelf-life of the product (Di Lecce, Bendini, Cerretani, Bonoli-Carbognin, & Lercker, 2006; Gomez-Alonzo, Mancebo-Campos, Salvador, & Fregapane, 2007; Servili & Montedoro, 2002). The phenolic content in VOO is the result of complex interactions among several factors, including cultivar, olive ripening degree, climate, and processing technology (Beltràn et al., 2005; Bonoli et al., 2004; Nergiz & Engez, 2000; Olivares-Lopez et al., 2007; Romero et al., 2002; Servili et al., 2007).

Table 1 reports the evolution of the total phenolic content of the F/L and Cor oils as determined through spectrophotometry. The F/L oil blend showed a lower content of total phenolics with respect to the Cor oil (70% less, on average).

The concentrations of the total phenols were positively correlated with malaxation temperature (Table 1) (F/L, $R^2 = 0.887$; Cor, $R^2 = 0.992$). At T_0, for the malaxation temperature of 25 °C, the fresh F/L blend showed lower total phenols, by 36% and 41%, than seen for the oils obtained at 35 °C and 45 °C, respectively. Similarly for the fresh Cor oils, with again higher total phenols of 14% and 28% at 35 °C and 45 °C, respectively. Thus, an increase in malaxation temperature leads to a greater release of the olive phenols from the solid parts of the fruit into the oil phase. These increases in phenol concentrations in the F/L blends were higher when the olive paste was heated from 25 °C to 35 °C (36% more phenolic compounds), while in the Cor oils, the phenolic contents were higher when the olive paste was heated from 35 °C to 45 °C (17% more phenolics). These findings are in agreement with the results obtained in other studies (Lercker, Frega, Bocci, & Mozzon, 1999; Servili, Baldioli, & Montedoro, 1994; Solinas, Di Giovacchino, & Mascolo, 1978).

For storage time effects here, the total phenolic content increased during the first six (T_6) and nine (T_9) months in the F/L oils. In the Cor oils, the total phenolic concentrations increased by 14%, 25% and 37%, at 25 °C, 35 °C and 45 °C, respectively, after 12 months storage. These trends can be explained by considering the two different stages of this experimental plan: oil extraction, and oil storage. The extraction rate of phenols from the solid part into the oil during malaxation is limited by the processing temperature in the case of Cor oils, the phenolic contents of which were much higher than for the F/L oils. Conversely, the limiting factor was the low concentration of phenolics in the case of the F/L blends, as shown in Figs. 3 and 4. Later, the lysis of the complex phenols during the oil storage led to a higher content of low molecular weight phenolics, as discussed further below.

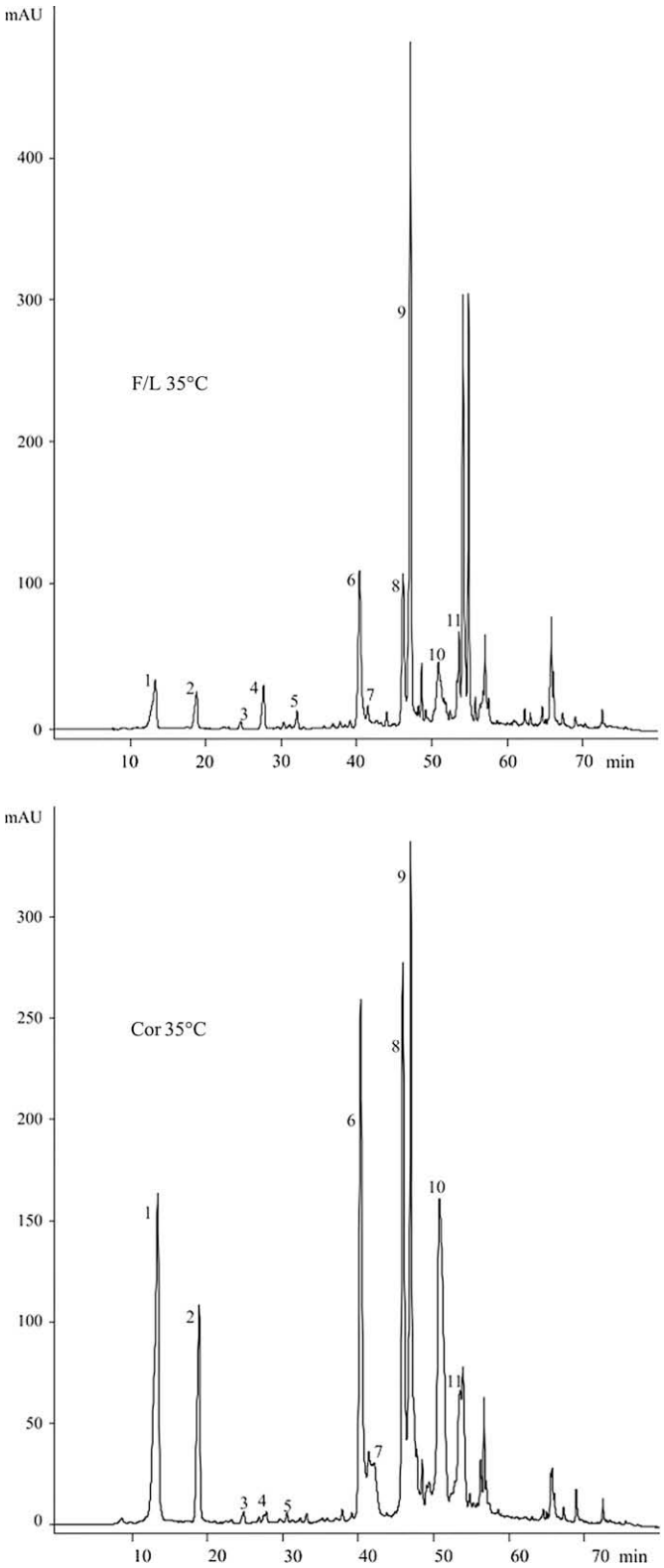

Fig. 7. Phenolic profile ($\lambda = 280$ nm) of the F/L and Cor oils at 35 °C (T_9): 1, 3,4-DHPEA; 2, *p*-HPEA; 3, vanillic acid; 4, vanillin; 5, 3,4-DHPEA-Ac; 6, 3,4-DHPEA-EDA; 7, 3,4-DHPEA-EDA-Ox; 8, *p*-HPEA-EDA; 9, *p*-HPEA-EDA-Ox + LA; 10, 3,4-DHPEA-EA; 11, LA.

The oxidative stabilities of the oils were measured as the induction time in response to forced oxidation, and they are shown in Table 1. At T_0, the induction periods of the Cor oils were two-fold those of the F/L oil blends, showing a higher stability of the Cor oils with respect to the F/L oils. Interestingly, the oxidative stabilities increased with increased malaxation temperature, with the correlation between induction time and malaxation temperature higher for Cor ($R^2 = 0.987$) than for F/L ($R^2 = 0.818$) oils, as shown in Fig. 3. This trend is very clear, even if the two oil batches are clearly differentiated due to their different phenolic compositions: the Cor oils (with high phenolic content) showed greater stability than the F/L oils (with low phenolic content). The Cor oils also showed higher oleic/linoleic ratios as compared to the F/L blends, as shown in Table 2. Thus, the induction period was not only well correlated with the total phenolic content for all of the oils (Fig. 4) ($R^2 = 0.751$), but also well correlated with the oleic/linoleic ratios ($R^2 = 0.875$). The presence of two double bonds in the fatty acid chain increases the oxidation rate by about 10-fold with respect to oleic acid.

However, a decrease in the oxidative stability during storage was seen for all of the samples, and especially in the first three months of storage of the F/L oils and six months of storage of the Cor oils (Fig. 5), as reported in Table 1.

Discordant results can be found in the literature on the effects of malaxation temperature on the total phenolic content of VOO. As reported by Parenti, Spugnoli, and Cardini (2000) and Ranalli, Malfatti, Lucera, Contento, and Sotiriou (2005), an increase in the malaxation temperature can lead to a reduction in the total phenolic content of oils obtained using small laboratory equipment. However, investigations conducted using industrial processing plants have provided the opposite results (Cerretani, Bendini, Gallina Toschi, Lercker, & Rotondi, 2006; Servili et al., 2004) and are in agreement with the present study. These differences appear to be due to the different experimental conditions, and particularly to the higher mass/surface ratio of the olive paste being processed in a large scale industrial processing plant. In these cases, the olive paste is less affected by the activities of oxidative enzymes, as its contact with the air is reduced to a minimum (Di Giovacchino et al., 2002).

3.4. Characterization of phenolic compounds by HPLC–DAD–MSD²

The HPLC analysis of the phenolic composition of the F/L and Cor oils obtained at the three different malaxation temperatures and stored for 12 months is reported in Table 3. The results are different with respect to the 'total phenolics' above due to the different quantitation procedures. The 'total phenols' above are the result of a colorimetric procedure for Folin–Ciocalteu reagent, and the data were converted into gallic acid equivalents. Instead, the phenolics determined by HPLC were quantified using standard substances with similar UV absorbances. However, the total phenolic content calculated as the sum of the peaks detected with HPLC in the six samples of fresh oil (T_0) increased with increasing malaxation temperature, consistent with the results obtained using the Folin–Ciocalteu method.

The HPLC profile was recorded at a wavelength of 280 nm (Figs. 6 and 7). HPLC–DAD–MS/MS was used to confirm the identification of the phenolic acids, phenolic alcohols, secoiridoids and flavones. The significantly different concentrations of each phenolic compound among the different samples were calculated using Tukey's test. The trend of the main classes (low molecular weight phenolics, secoiridoids and flavones) of the oil phenolics is shown in Fig. 8.

3.4.1. Low molecular weight phenolics

As shown in Fig. 8, the trend seen for the low molecular weight phenolics (MW < 170) was similar in both of the oil types (F/L and Cor), although they were characterized by different phenolic concentrations: the content of this phenolic class increased at the end of the 12-month storage period.

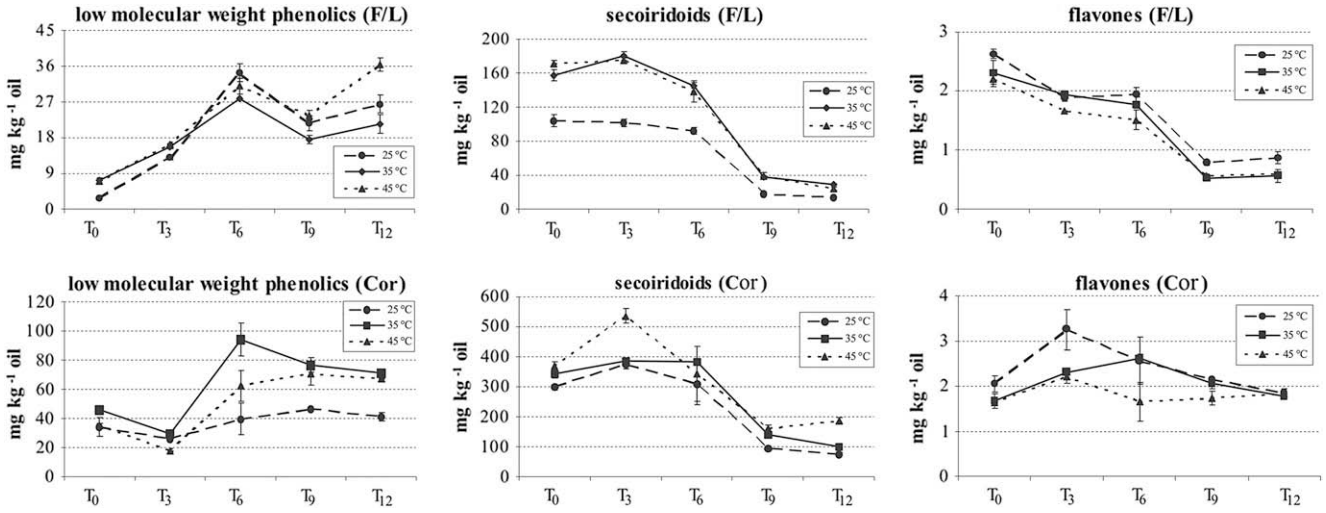

Fig. 8. Trends of the phenolic classes of the VOO samples during the 12 months of storage.

Hydroxytyrosol (3,4-dihydroxyphenylethanol, 3,4-DHPEA), and tyrosol (*p*-hydroxyphenylethanol, *p*-HPEA) were the main phenolic alcohols. Their concentrations were low in the fresh oils, but increased during storage, as reported in previous studies (Brenes, Garcia, Garcia, & Garrido, 2001; Servili et al., 1999). This increase appears to be due to the lysis of the secoiridoids, such as the dialdehydic forms of decarboxymethyl elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA) and to *p*-HPEA (*p*-HPEA-EDA), and oleuropein aglycon (3,4-DHPEA-EA); these compounds are precursors of 3,4-DHPEA and *p*-HPEA, respectively. In the F/L oils, the 3,4-DHPEA and *p*-HPEA contents were lower than for the Cor oils at the three different malaxation temperatures. Malaxation temperature did not affect the 3,4-DHPEA contents in the fresh oils (T_0), except for the F/L oils at 25 °C (here, the 3,4-DHPEA content was below the detection limit of 1.5 mg kg^{-1}). As reported in Table 3, the concentrations of 3,4-DHPEA increased during the first six/nine months of storage.

At time T_0, the F/L and Cor oils obtained at 35 °C contained higher concentrations of *p*-HPEA (3.61 and 27.1 mg kg^{-1} of oil, respectively) compared to the oils obtained at 25 °C and 45 °C. The contents of vanillic acid and vanillin did not exceed 1.66 mg kg^{-1} of oil, and they were positively correlated with malaxation temperature.

3.4.2. Hydroxytyrosol acetate (3,4-DHPEA-Ac)

The contents of hydroxytyrosol acetate were higher in the F/L oils than in the Cor oils, with a trend that was more similar to that of the secoiridoids and flavones: it decreased during storage of the F/L oils. Hydrolysis of the acetic ester occurred during the storage, leading to the free alcohol (3,4-DHPEA), which indeed increased during the oil conservation.

3.4.3. Secoiridoids

The trend shown by the secoiridoids is shown in Fig. 8. The concentration of secoiridoids in the fresh oils was positively related to the malaxation temperature and also decreased during storage, particularly between six and nine months after the oil production.

In the freshly obtained F/L oils, 3,4-DHPEA-EDA and *p*-HPEA-EDA increased from 6.0 to 49.9 mg kg^{-1} and from 73.5 to 96.0 mg kg^{-1}, respectively, when the malaxation temperature increased from 25 °C to 45 °C. The same trend was seen for the Cor oils, where the 3,4-DHPEA-EDA and *p*-HPEA-EDA contents were higher than those for the F/L blends: their concentration increased from 39.5 to 118 mg kg^{-1} and from 101 to 152 mg kg^{-1}, respectively (Table 3 and Fig. 6).

Over the storage period, the contents of 3,4-DHPEA-EDA and *p*-HPEA-EDA decreased for all of the oils, as shown in Table 3. In the F/L oils obtained at 25 °C and 45 °C, the 3,4-DHPEA-EDA contents had already decreased during the first 3 months, while in the F/L oil produced at 35 °C, the contents of 3,4-DHPEA-EDA only decreased starting from three months of storage (T_3). The F/L oils obtained at 35 °C and 45 °C showed similar trends with regard to *p*-HPEA-EDA: an increase was seen at time T_3 (3 months), which then decreased to 8.93 and 7.30 mg kg^{-1} at T_{12}, respectively. The contents of 3,4-DHPEA-EDA halved after 12 months of storage in all of the Cor oils. In contrast, the *p*-HPEA-EDA contents of the Cor oils was stable over the first three months (T_0–T_3), but decreased later in all of the samples, and particularly in the oils obtained at 25 °C and 35 °C.

The evolution of 3,4-DHPEA-EA and ligstroside aglycon (LA) was different according to the oil type (F/L or Cor) (Table 3). In the F/L oils, the concentrations of 3,4-DHPEA-EA and LA were lower than in the Cor oils. In the freshly obtained F/L oils (T_0), the contents of 3,4-DHPEA-EA increased with malaxation temperature by 33% and 41% at 35 °C and 45 °C, respectively, with respect to the F/L oil obtained at 25 °C. The same compounds showed an opposite trend in the Cor oils: the concentration of 3,4-DHPEA-EA decreased with malaxation temperature by 9% and 35% at 35 °C and 45 °C, respectively. The same trend was observed for LA, which appeared only after nine and 12 months, and did not exceed 5.6 mg kg^{-1} in all of the F/L oils. However, in all of the samples, 3,4-DHPEA-EA and LA decreased during storage.

In the Cor oils, the carboxymethyl moiety of elenolic acid was subjected to cleavage during the first three months; thus, the increase in 3,4-DHPEA-EDA and *p*-HPEA-EDA occurred to the expense of 3,4-DHPEA-EA and LA, respectively. The oxidation products of 3,4-DHPEA-EDA and *p*-HPEA-EDA appeared after nine months, in the samples of both F/L and Cor oils, with lower concentrations in the F/L oils than in the Cor oils.

3.4.4. Flavones

The flavones (luteolin and apigenin) showed a trend that varied according to oil type. In the F/L oils, the contents of the flavones decreased, presumably due to a lower concentration of total phenolics, leading to their degradation to oxidation products. In the Cor oils, the contents of the flavone were stable during storage. In both oil types, methoxyluteolin was present only at T_9 and T_{12}, in all of the samples.

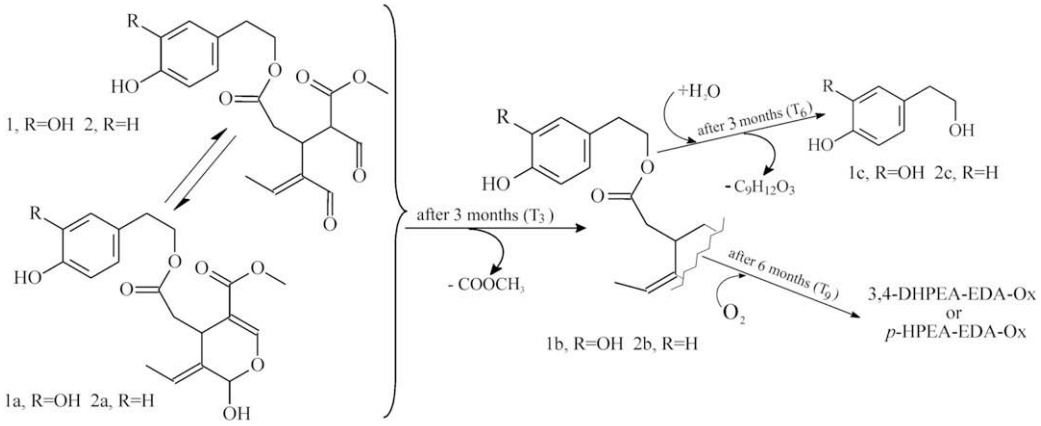

Fig. 9. Evolution of the secoiridoids during the storage of the VOO. 1, dialdehydic form of 3,4-DHPEA-EA; 1a, hydroxy form of 3,4-DHPEA-EA; 1b, 3,4-DHPEA-EDA; 1c, 3,4-DHPEA; 2, dialdehydic form of LA; 2a, hydroxy form of LA; 2b, *p*-DHPEA-EDA; 2c, *p*-DHPEA.

4. Conclusions

VOO obtained at a processing temperature lower than 27 °C ('first cold pressing' or 'cold extraction' according to EU legislation) did not show higher oxidative stability or sensory qualities than VOO obtained at 35 °C. The oils obtained at 35 °C were considered to be of good quality by the panelists, whereas the oils obtained at 45 °C were rejected because they contained a 'heated or burnt' off-flavour.

These results were obtained from two oils characterized by different chemical properties at time T_0: the F/L blends had higher acidity and peroxide levels, but lower phenolic content and a lower oleic/linoleic ratio than the Cor oil. However, both of the oils showed the same trends during storage: in the freshly obtained oils (T_0), the oxidative stability was positively correlated with phenolic content and with malaxation temperature. Moreover, the oils obtained at the higher temperatures did not exceed any of the legal chemical quality parameters by the end of the storage period.

As a consequence of the protective antioxidant effects of the phenolics (Meteos, Dominguez, Espartero, & Cert, 2003; Owen et al., 2000; Visioli, Bellomo, & Galli, 1998), the peroxide levels of the oils were not related to the processing temperatures, as one would expect considering that the oxidation rate is.

As shown in Fig. 9 and based on the results reported in Table 3, the first three months of storage resulted in the decarboxymethylation of the secoiridoids, whereas the lysis of the ester bond lasted beyond after six months. The oxidation products of the secoiridoids were detectable only after nine months of storage.

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