Eur Food Res Technol (2007) 225:617–625
DOI 10.1007/s00217-006-0456-0

ORIGINAL PAPER

Partition of phenolic compounds during the virgin olive oil industrial extraction process

Luz-Stella Artajo · Maria-Paz Romero · Manuel Suárez · Maria-José Motilva

Received: 10 May 2006 / Revised: 21 July 2006 / Accepted: 29 July 2006 / Published online: 15 September 2006

Abstract Phenolics have been considered an important group of natural antioxidants. The type and quantity of phenolics vary in olive matrices (leaves, fruit, stones, seeds, and paste). However, the relationships linking the products from the olive oil extraction process are poorly studied. This manuscript deals with the partition of phenolic compounds during olive oil production season at three times. Samples were taken during malaxation to determine the phenolic content in the olive paste at 0, 15, and 45 min. The wet pomace and oil phases were analyzed and the phenolics quantified in terms of kilogram of olive paste. Malaxation time had an important effect on the alcohols and secoiridoids. Hydroxytyrosol and tyrosol decreased, and their hydrophilic character was proved through their presence in the wet pomace and wastewater. Oleuropein and ligstroside degradation results in the formation of secoiridoid derivatives, mainly the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA). A molar transfer index (MTI) was established between the 3,4-DHPEA-EDA in the olive paste and olive oil (as the liquid phase) and wet pomace (as the solid phase). An increasing trend was observed for the molar transference to virgin olive oil with the advance of the crop season.

Keywords Phenolic partition · Phenolic compound · Olive oil · Two-phase system · Virgin olive oil extraction

Introduction

Biophenols, a wide range of secondary metabolites from the shikimate pathway and phenylpropanoid metabolism, play an important role in human health. Interest in phenolic compounds has increased over recent years, given their potential antioxidant activity and possible effects against degenerative illness. In fact, among the hydrosoluble compounds, polyphenols in olive fruit are highly significant due to their wide range of biochemical and pharmaceutical effects including anticarcinogenic, antiatherogenic, and antimicrobial properties [1–3].

It is widely known that the composition of the phenolic fraction in olive oil depends on the cultivar, climatic conditions during growth, degree of maturation, and the technology used for oil extraction [4, 5]. The degree of olive ripening has a notable effect on the quantity of *o*-diphenols and secoiridoid compounds [6, 7]. Moreover, several studies have focused on the study of variables, such a time, temperature and the use of enzymes during olive oil processing and their effect on the quality and nutritional properties of the oil [8–11]. Crushing and malaxation operations influenced the total phenolic and *o*-diphenols contents in virgin olive oil [4–6].

Since the beginning of the 1970s, the technology for olive oil extraction process has progressed significantly with the introduction of the three-phase system and, later, the two-phase system. The resulting solid phases (pomace and wet pomace) and liquid phases (oil and wastewater) can be separated in a continuous process. The continuous two-phase process is the most widely accepted extraction process in the Spanish olive oil industry, as shown by its use in 90% of the olive mills, achieving a very wet pomace with a water content that varies between 65 and 70%. In contrast, the common extraction system in the olive oil industry in Italy consists of three-phase decanters with the possibility of adding a small quantity of water [4, 12, 13].

Interest has also focused on the influence of the extraction methodologies on the sensory attributes, volatile compounds,

L.-S. Artajo · M.-P. Romero · M. Suárez · M.-J. Motilva (✉)
Food Technology Department, CeRTA-TPV, University of Lleida,
Av/Alcalde Rovira Roure 191,
25198 Lleida, Spain
e-mail: motilva@tecal.udl.es

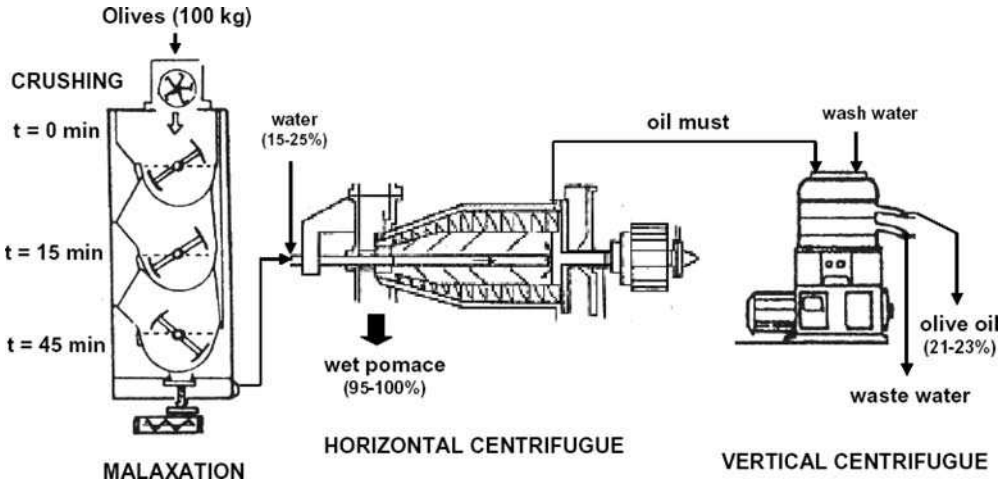

Fig. 1 Virgin olive oil industrial extraction process (two phases)

and natural antioxidants such as the phenolic compounds [12, 14, 15].

Different studies have continued to report on the composition of the extra virgin olive oil and its nutritional properties. Previous works reported a higher antioxidant power for the phenolic compounds naturally occurring in extra virgin olive oil [16–19]. Olive oil residues were tested for their composition of simple phenolic compounds as a function of the extraction systems [20]. Moreover, prior studies carried out by our research group proved that an increase in the ripening index of the olive fruit implied an increase in the hydroxytyrosol concentration in pomace. Similarly, the flavonoids showed a clear increase over the different phases (olive paste, pomace, oil, and wastewater) [21]. The water status of the olive trees affected the phenol synthesis in the olive fruit, and consequently the phenol content of the olive paste, more than the partitioning of the phenolic compounds during the olive oil extraction process [22]. However, there is limited information available about the partitioning of the phenolic compounds during the whole virgin olive oil industrial extraction process.

The main objective of this study was to investigate the effect of the malaxing process on the phenolic profile of the olive paste. Additionally, the partition of the phenolic compounds from olive paste to virgin oil was evaluated, together with the by-products resulting from the olive oil extracting process at an industrial level, considering three different harvest periods during the crop season.

Materials and methods

Samples

The experimental work was performed during the olive oil crop season (November 2004–January 2005) in an olive oil mill from the Les Garrigues production area of Catalonia (Spain) at three times during the harvest period (First period: November 1, 2004, middle period: December 10, 2004, and final period: January 5, 2005). A two-phase continuous cycle modular machinery (Pieralisi system, Italy) was used for extracting olive oil. The harvested olives were washed with mains water and the leaves were removed in order to avoid them adversely affecting the flavor of the resulting product and damaging the equipment used [4]. Olives from the Arbequina cultivar were crushed by metal crushers and the paste was mixed in a tank kneader (malaxation step) with a horizontal shaft (spiral steel mixing blades) equipped with a heating jacket (Fig. 1). This operation was carried out at 30 °C for 1 h. The paste was then sent to a decanter with a maximum water flow rate of 35,000 L/h and 15–25% of water was added in order to induce the extraction of the oil during the horizontal centrifugation, depending on the initial moisture content of the olive paste. This operation was performed at "2412 $\times$ g" and two separate products were obtained after a second centrifugation using a vertical separator, these being a sloppy paste of olive pulp with pits (wet pomace) and oil must (edible oil and vegetable water).

Homogenized olive paste samples were obtained from the semi-cylindrical vat at 0, 15, and 45 min. Samples of wet pomace and olive oil must were taken from the output of the horizontal centrifuge after 1 h in order to obtain the fractions from the same batch. Then, liquid nitrogen was added to every sample to avoid oxidative damage and each sample was kept in cold storage at -40 °C before analysis. Olive oil and wastewater samples were selected from the vertical centrifuge. Sampling was performed in triplicate.

Olive paste and wet pomace

Moisture content

Moisture was measured following the UNE Standard Spanish method (Asociación Española de Normalización y Cer-

tificación) [23]. About 10 g of olive paste and wet pomace were weighed, then dried for 24 h at 105 °C, cooled for 30 min in a desiccator and reweighed.

Oil content

Dried samples of olive paste and wet pomace were measured in duplicate with a NMS 100 Minispec NMR Analyzer (Bruker Analytik, Silberstreifen, Germany) using the ExpSpel Version 2.10 software. The results were expressed as a percentage of oil obtained with respect to the raw material.

Extraction of phenolic compounds

The phenolic compounds were extracted from the olive paste and wet pomace as reported by our research group [21] with modifications. A 10 g sample was mixed with 80 ml of aqueous ethanol 80% containing sodium methabisulphide (400 mg/l). The solution was homogenized with a Polytron for 2 min and centrifuged at "1685 × *g*" for 5 min and filtered under vacuum conditions. Then, the ethanolic extract was evaporated until dryness in a rotary evaporator under vacuum conditions at 32 °C to a volume of 1 ml (syrup consistence). A second alcoholic extraction was performed with 120 ml of acidified methanol (pH 2.5) and 40 ml of *n*-hexane. The final methanol extract was rotary evaporated at 32 °C to dryness and the phenolic extract was diluted in methanol grade HPLC. The extraction was done in triplicate. The phenolic compound extracted from the olive paste and wet pomace was dissolved in 1 ml of methanol and analyzed by HPLC.

Olive oil and oil must

Extraction of phenolic compounds

The extraction of phenolic compounds from oil must and olive oil was done following the method described in a previous work [7]. Analyses were done in triplicate. The resulting extract was filtered through a 0.45-μm filter (Whatman Inc., Clifton, NY), and dissolved in 1 ml of methanol before HPLC analysis.

Evaluation of oxidative stability

Stability is expressed as the oxidation induction time (hours) measured with a Rancimat 679 apparatus (Metrohom Ltda, Switzerland) using an oil sample of 2.5 g warmed to 120 °C and 120 l/h air flow. The time taken to reach a fixed level of conductivity was measured. Each assessment was done in triplicate.

HPLC analysis of phenolic compounds

The phenolic fraction extracted from the olive paste, wet pomace, and oil was analyzed by HPLC. The wastewater was centrifuged, filtered through the 0.45-μm filter, and injected into the chromatograph. The Waters system (Milford, USA) included a pump (600 E), a column heater, an autosampler (717 plus) equipped with a 20-μl loop injector and a photodiode array detector (996). Separation was achieved on a 5 μm, 15 cm × 4.6 mm i.d., Inertsil ODS-3 column (GL Sciences Inc.) equipped with a 5 μm, 1 cm × 4.6 mm i.d., Spherisorb S5 ODS-2 precolumn (Technokroma, Barcelona, Spain). The mobile phases were degassed under vacuum using continuously sparged with high-purity helium during analysis. Water/acetic acid (100:02 v/v) was used as solvent A and methanol as solvent B. Solvent A was held isocratically at 95% for 2 min, then decreased to 75% at 10 min followed by further linear reduction to 60% at 20 min, then decreased to 50% at 30 min, and reduced to 0% at 40 min with 5 min isocratic time, followed by a strong linear ramping to 95% at 55 min and then held constant for 5 min. Empower software (Milford, USA) was used to operate the system and the output of the photodiode array detector was monitored at 240, 280, and 339 nm. Each phenolic compound was tentatively identified by its retention and UV spectrum characteristics. The quantification was performed by using a four-point regression curve on the basis of the reference compounds.

Reference compounds

Commercial standards from the following sources were used without further purification: apigenin 7-*O*-glucoside; hydroxytyrosol (3,4-DHPEA); luteolin; luteolin-7-*O*-glucoside, oleuropein, tyrosol (*p*-HPEA), and vanillin from Extrasynthese (Genay, France); caffeic acid; *o*-coumaric acid; vanillic acid from Fluka Co. (Buchs, Swiss); homovanillic acid from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Non-commercial phenolic compounds were obtained from phenolic extracts from virgin olive oil by semi-preparative HPLC [16].

Molar Transfer Index (MTI)

Molar transfer indexes (MTIs) were established in order to compare the partitioning of the more important secoiridoid derivative of virgin olive oil (the dialdehydic form of elenolic acid linked to hydroxytyrosol or 3,4-DHPEA-EDA) from olive paste to olive oil (MTIp), and the partitioning of the 3,4-DHPEA-EDA from olive paste to wet pomace (MTIw) at the three different periods over the olive oil production season.

MTIp = MOV/MOP, where MOP is the molar concentration of the 3,4-DHPEA-EDA in 1 kg of olive paste at the end of the malaxation step ($t = 45$ min) and MOV is the molar concentration of the 3,4-DHPEA-EDA in the olive oil extracted from 1 kg of olive paste.

MTIw = MWP/MOP, where MOP is the molar concentration of the 3,4-DHPEA-EDA in 1 kg of olive paste at the end of the malaxation step ($t = 45$ min) and MWP is the molar concentration of the 3,4-DHPEA-EDA in the wet pomace obtained from 1 kg of olive paste.

Results and discussion

Effect of processing on the phenolic compound partition

Tables 1, 2 and 3 respectively show the phenolic compounds identified in the solid and liquid phases obtained during the industrial oil extraction process in the three olive oil production periods. Hydroxytyrosol, hydroxytyrosol derivative, and tyrosol, the most important phenolic alcohols occurring in olives, were detected in significant amounts in the olive paste. With regard to the secoiridoid group, it can be noted that after the crushing operation, the ligstroside (secoiridoid characterized by an exocyclic 8,9-olefinic functionality) was the predominant compound in the olive paste during the three samplings, whereas oleuropein showed a decreasing trend not only during the malaxation operation but also over the three samplings. The degradation of oleuropein and related compounds could be performed by two main routes: enzymatic cleavage by esterases or activation of the β-glucosidades. The theory of this previous cleavage by specific esterases with the consequent rise of either elenolic acid glucoside or demethyloleuropein has been proposed. However, it is generally accepted that crushing is the first step toward the activation of the endogenous β-glucosidases found in the olive fruit, resulting in a multi-phased system. Demethyloleuropein, derived from esterase activity, may act as a substrate and a solid stable aglycone product is then formed [1].

The most important secoiridoid derivative, 3,4-DHPEA-EDA (the dialdehydic form of elenolic acid linked to hydroxytyrosol) was detected in a significant quantity in the olive paste system showing a decrease with the increase in the malaxation time. This could confirm the previous action of β-glucosidases in the crushing, even though the other secoiridoid derivatives were not detected as partition products. The hydrocinnamic derivate, verbascoside, was also found in the olive paste. Some authors have reported its presence in peel, pulp, and seed matrices [24–28]. Flavonoids, such as luteolin 7-*O*-glucoside and rutin, have only been reported in olive peel. This could explain that the mechanical operation results in a transference of these compounds to both the olive paste and wet pomace phases. To sum up, a wide range of phenolic compounds have been reported in olive paste and wet pomace, including simple phenolic alcohols and acids, phenolic glucosides, phenolic oleosides, and flavonoids. The olive paste could be considered a dynamic state in which biophenols are partitioned into different phases according to their affinity for water or oil.

In the liquid phases, olive oil is dominated by secoiridoid derivatives, followed by flavonoids and phenolic alcohols. The presence of the secoiridoid derivatives is an indicator of the above-mentioned degradation pathways for the phenolic oleosides shown in the solid phases. It was assumed that some phenolic compounds found in olive oil are naturally occurring as a result of processing. In the two-phase extraction system, water was added (ranging from 15 to 25%) at the end of malaxation step. This could contribute to a degradation of the oleoside compounds, although the mechanism in terms of quantity for the transformations is far from being elucidated completely. The final products of the different mechanisms proposed in previous works [29, 30] occur at a lipidic/water interface, resulting in complicated isomerisations and equilibrium. Moreover, the partition of antioxidants in the two different phases (wet pomace and oil must) depend on their relative affinity toward solid and liquid phases [21, 31].

Effect of the period of the production season on the partitioning of phenolic compounds

The phenolic compound contents of olive paste from the three periods of the production season, (first, middle, and final) and their partitioning into wet pomace and oil are shown in Tables 1, 2 and 3, respectively. The results are expressed as milligram per kilogram olive paste immediately after crushing (time = 0 min) taking into account both mass and component balances during the oil extraction process. The concentration of the different phenolic groups happening in the solid and liquid phases decreased significantly in the second and third samplings in relation to the first season period, maybe as a consequence of the advanced ripening stage of the olive fruit, especially at the end of the harvesting period. In olive paste, the matrix obtained after the crushing step, a significant amount of phenolic alcohols were detected and hydroxytyrosol (3,4-DHPEA) and its derivatives were the most abundant at the beginning of the malaxation operation (Tables 1a, 2a and 3a). The 3,4-DHPEA derivatives found in olive paste phase are probably related to the crushing operation, which allows the transformation of hydroxytyrosol compounds. The increase in the total simple phenols in the wet pomace from the first sampling should be noted and the hydrophilic character of the simple phenols could explain their affinity for the aqueous phase with an increase around of 17% in relation to the olive paste (Table 1a). In the three sampling periods, the oil phases (oil must

Table 1 Partition of phenolic compounds (expressed as milligram per kilogram of olive paste) during the olive oil extraction process: first period of the production season

	Malaxation (time) Olive paste			Horizontal centrifuge Intermediate products		Vertical centrifuge Final Products	
	$t = 0$ min	$t = 15$ min	$t = 45$ min	Wet pomace	Oil must	Olive oil	Wastewater
(a) Phenolic alcohols[a]							
3,4-DHPEA derivate	31.9 ± 1.8	29.5 ± 8.3	21.8 ± 2.6	27.0 ± 1.4	nd	nd	0.0003 ± 7.58 e^{-6}
3,4-DHPEA	95.6 ± 5.1	59.0 ± 9.9	48.6 ± 1.4	114 ± 9	0.032 ± 0.003	0.023 ± 0.006	0.0010 ± 9.61 e^{-5}
3,4-DHPEA derivate	87.0 ± 5.7	129 ± 19	116 ± 11	119 ± 5	nd	nd	0.0006 ± 7.88 e^{-5}
p-HPEA	88.1 ± 4.7	77.9 ± 12.7	71.6 ± 8.2	94.7 ± 7.5	0.084 ± 0.012	0.032 ± 0.012	0.0010 ± 1.00 e^{-4}
Total	303	296	258	355	0.115	0.055	0.0029
Secoiridoids							
Demethyloleuropein	359 ± 64	330 ± 49	172 ± 19	170 ± 10	nd	nd	nd
Oleuropein	343 ± 16	154 ± 13	141 ± 10	nd	nd	nd	nd
Demethyl-ligstroside	348 ± 91	271 ± 24	236 ± 27	356 ± 30	nd	nd	nd
Ligstroside	895 ± 42	762 ± 39	761 ± 23	nd	nd	nd	nd
Total	1944	1516	1310	526	nd	nd	nd
Secoiridoid derivates							
3,4-DHPEA-AC	nd	nd	nd	nd	5.5 ± 0.8	8.3 ± 1.1	nd
3,4-DHPEA-EDA	3690 ± 153	3788 ± 140	3259 ± 93	3375 ± 62	41.2 ± 4.0	83.1 ± 5.2	tr
p-HPEA-EDA	nd	nd	nd	nd	16.1 ± 1.1	15.8 ± 2.1	nd
p-HPEA-EA	nd	nd	nd	nd	10.5 ± 2.1	9.9 ± 0.9	nd
3,4-DHPEA-EA	nd	nd	nd	nd	18.3 ± 2.0	19.0 ± 1.3	nd
ME 3,4-DHPEA-EA	nd	nd	nd	nd	1.2 ± 0.5	1.2 ± 0.2	nd
Total	3690	3788	3259	3375	92.8	137	nd
Elenolic acid[b]	nd	nd	nd	nd	24.8 ± 1.0	22.2 ± 1.8	nd
Verbascoside	168 ± 3	184 ± 38	182 ± 4	179 ± 6	nd	nd	nd
(b) Phenolic acids and derivates[b]							
Vanillic acid	53.9 ± 1.8	45.4 ± 2.3	42.9 ± 3.1	45.4 ± 2.3	0.064 ± 0.005	0.042 ± 0.002	0.0003 ± 3.66 e^{-6}
Homovanillic acid	109 ± 17	98.4 ± 3.1	80.5 ± 8.0	98.4 ± 3.1	0.033 ± 0.004	0.021 ± 0.004	0.0009 ± 3.70 e^{-5}
Vanillin	34.4 ± 1.3	20.3 ± 3.0	23.0 ± 1.1	33.3 ± 0.9	0.118 ± 0.010	0.097 ± 0.002	0.0002 ± 5.30 e^{-5}
Total	197	164	146	177	0.215	0.160	0.0014
Flavonoids							
Luteolin-7-O-G	30.6 ± 1.9	24.9 ± 2.9	23.1 ± 4.7	27.8 ± 2.8	nd	nd	nd
Rutin	91.3 ± 4.8	92.1 ± 13.6	80.2 ± 7.5	89.0 ± 3.3	nd	nd	nd
Apigenin-7-O-G	12.3 ± 3.5	11.9 ± 2.7	11.1 ± 1.4	9.0 ± 2.0	nd	nd	nd
Luteolin	113 ± 2	60.4 ± 13.5	66.4 ± 1.9	96.2 ± 3.4	0.68 ± 0.07	0.71 ± 0.10	nd
Apigenin	10.0 ± 1.3	9.1 ± 2.0	8.7 ± 1.7	9.3 ± 0.1	0.31 ± 0.03	0.30 ± 0.05	nd
Total	245	198	190	231	0.99	1.01	nd
Lignans							
Acetoxypinoresinol + pinoresinol	nd	nd	nd	nd	24.8 ± 2.2	22.7 ± 2.4	nd

Values are the mean ± SD of four experiments. nd, not detected; tr, traces.

[a] 3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol; 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; *p*-HPEA-EA, aldehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropein aglycone; ME 3,4-DHPEA-EA, methylated form of oleuropein alycone; Luteolin-7-O-G, luteolin-7-*O*-glucoside; Apigenin-7-O-G, apigenin-7-*O*-glucoside.

[b] Not phenolic compound.

Table 2 Partition of phenolic compounds (expressed as milligram per kilogram of olive paste) during the olive oil extraction process: middle period of the production season

	Malaxation time Olive paste			Horizontal centrifugation Intermediate products		Vertical centrifugation Final products	
	$t = 0$ min	$t = 15$ min	$t = 45$ min	Wet pomace	Oil must	Olive oil	Wastewater
(a) Phenolic alcohols[a]							
3,4-DHPEA derivate	29.0 ± 3.0	43.3 ± 2.8	42.1 ± 3.5	24.0 ± 2.5	nd	nd	0.0013 ± 7.6 e^{-5}
3,4-DHPEA	43.2 ± 2.2	63.1 ± 4.2	68.8 ± 1.3	64.1 ± 1.3	0.011 ± 0.001	0.017 ± 0.001	0.0029 ± 8.1 e^{-5}
3,4-DHPEA derivate	109 ± 8.7	89.3 ± 2.1	91.9 ± 2.3	68.4 ± 0.8	nd	nd	0.0015 ± 9.8 e^{-5}
p-HPEA	61.2 ± 4.1	68.4 ± 4.3	75.5 ± 2.4	56.6 ± 3.5	0.137 ± 0.01	0.109 ± 0.005	0.0004 ± 3.3 e^{-4}
Total	243	264	278	213	0.148	0.126	0.0061
Secoiridoids							
Demethyloleuropein	337 ± 7	238 ± 16	243 ± 13	179 ± 8	nd	nd	nd
Oleuropein	94.8 ± 9.2	tr	tr	nd	nd	nd	nd
Demethyl-ligstroside	158 ± 7	133 ± 4	101 ± 5	101 ± 3	nd	nd	nd
Ligstroside	190 ± 4	nd	nd	nd	nd	nd	nd
Total	781	371	344	280	nd	nd	nd
Secoiridoid derivates							
3,4-DHPEA-AC	nd	nd	nd	nd	5.20 ± 0.05	7.60 ± 0.27	nd
3,4-DHPEA-EDA	1732 ± 88	502 ± 50	449 ± 32	472 ± 9	12.10 ± 0.07	25.70 ± 0.91	tr
p-HPEA-EDA	nd	nd	nd	nd	14.00 ± 0.13	13.50 ± 0.45	nd
p-HPEA-EA	nd	nd	nd	nd	9.20 ± 0.44	8.5 ± 0.45	nd
3,4-DHPEA-EA	nd	nd	nd	nd	nd	nd	nd
ME 3,4-DHPEA-EA	nd	nd	nd	nd	0.90 ± 0.06	0.70 ± 0.04	nd
Total	1732	502	449	472	41.4	56.0	nd
Elenolic acid[b]	nd	nd	nd	nd	13.1 ± 0.63	10.8 ± 1.19	nd
Verbascoside	120 ± 27	126 ± 3	129 ± 2	117 ± 6	nd	nd	nd
(b) Phenolic acids and derivates							
Vanillic acid	25.4 ± 2.1	20.5 ± 7.7	31.6 ± 2.4	23.3 ± 3.0	0.047 ± 0.004	0.031 ± 0.009	0.0008 ± 5.1 e^{-4}
Homovanillic acid	70.2 ± 18.6	53.0 ± 16.6	57.6 ± 3.9	36.9 ± 0.3	nd	nd	0.0015 ± 4.3 e^{-4}
Vanillin	16.4 ± 3.5	13.4 ± 0.9	14.1 ± 0.7	15.1 ± 0.6	0.088 ± 0.001	0.080 ± 0.001	0.0010 ± 2.5 e^{-4}
Total	112	86.9	103	75.3	0.135	0.111	0.0033
Flavonoids							
Luteolin-7-O-G	31.5 ± 5.2	32.5 ± 1.9	34.0 ± 1.0	25.5 ± 0.6	nd	nd	nd
Rutin	86.1 ± 18.8	52.0 ± 3.2	51.9 ± 1.6	37.2 ± 0.8	nd	nd	nd
Apigenin-7-O-G	9.7 ± 0.3	7.8 ± 0.4	8.3 ± 1.4	6.6 ± 0.2	nd	nd	nd
Luteolin	56.4 ± 10.2	85.3 ± 2.4	86.1 ± 1.2	64.7 ± 0.4	0.381 ± 0.004	0.648 ± 0.016	nd
Apigenin	2.6 ± 1.1	5.7 ± 0.3	5.7 ± 0.2	4.1 ± 0.1	0.275 ± 0.027	0.310 ± 0.030	nd
Total	186	184	186	138	0.656	0.958	nd
Lignans							
Acetoxypinoresinol + pinoresinol	nd	nd	nd	nd	26.7 ± 1.5	26.0 ± 2.3	nd

Values are the mean ± SD of four experiments. nd, not detected; tr, traces.

[a] 3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol; 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; *p*-HPEA-EA, aldehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropein aglycone; ME 3,4-DHPEA-EA, methylated form of oleuropein alycone; Luteolin-7-O-G, luteolin-7-*O*-glucoside; Apigenin-7-O-G, apigenin-7-*O*-glucoside.

[b] Not phenolic compound.

Table 3 Partition of phenolic compounds (expressed as mg per kg of olive paste) during the olive oil extraction process: Final period of the production season

	Malaxation Olive paste			Horizontal centrifugation Intermediate products		Vertical centrifugation Final products	
	$t = 0$ min	$t = 15$ min	$t = 45$ min	Wet pomace	Oil must	Olive oil	Wastewater
(a) Phenolic alcohols[a]							
3,4-DHPEA derivate	50.8 ± 9.7	49.5 ± 9.4	37.5 ± 1.4	23.7 ± 1.4	nd	nd	0.0027 ± 5.8 e^{-5}
3,4-DHPEA	38.2 ± 4.1	32.0 ± 0.6	36.0 ± 2.0	29.3 ± 6.0	0.012 ± 0.002	0.021 ± 0.001	0.0037 ± 9.6 e^{-5}
3,4-DHPEA derivate	66.7 ± 6.8	67.0 ± 0.7	63.3 ± 7.9	54.1 ± 6.2	nd	nd	0.0033 ± 5.4 e^{-4}
p-HPEA	52.8 ± 10.4	50.3 ± 2.2	52.5 ± 6.0	48.9 ± 2.3	0.057 ± 0.002	0.027 ± 0.003	0.0028 ± 6.7 e^{-4}
Total	209	199	189	156	0.069	0.048	0.0029
Secoiridoids							
Demethyloleuropein	179 ± 9	17.3 ± 7.7	144 ± 9	112 ± 9	nd	nd	nd
Oleuropein	tr	tr	tr	nd	nd	nd	nd
Demethyl-ligstroside	nd	nd	nd	nd	nd	nd	nd
Ligstroside	nd	nd	nd	nd	nd	nd	nd
Total	179	177	144	112	nd	nd	nd
Secoiridoid derivates							
3,4-DHPEA-AC	nd	nd	nd	nd	0.20 ± 0.08	0.30 ± 0.01	nd
3,4-DHPEA-EDA	329 ± 16	266 ± 13	145 ± 9	122 ± 6	2.92 ± 0.10	1.92 ± 0.02	tr
p-HPEA-EDA	nd	nd	nd	nd	10.3 ± 0.5	10.5 ± 0.2	nd
p-HPEA-EA	nd	nd	nd	nd	5.1 ± 0.2	7.5 ± 0.2	nd
3,4-DHPEA-EA	nd	nd	nd	nd	nd	nd	nd
ME 3,4-DHPEA-EA	nd	nd	nd	nd	0.70 ± 0.04	0.23 ± 0.03	nd
Total	329	266	145	122	18.7	20.7	
Elenolic acid[b]	nd	nd	nd	nd	16.6 ± 2.1	13.0 ± 1.2	nd
Verbascoside	74.5 ± 4.3	68.7 ± 3.8	44.9 ± 1.9	20.7 ± 1.9	nd	nd	nd
(b) Phenolic acids and derivates							
Vanillic acid	26.4 ± 2.3	19.9 ± 0.7	24.3 ± 6.9	22.2 ± 5.0	0.031 ± 0.002	0.042 ± 0.002	0.0017 ± 3.7 e^{-4}
Homovanillic acid	66.6 ± 4.3	42.4 ± 4.8	23.6 ± 0.6	34.6 ± 7.5	nd	nd	0.0023 ± 6.4 e^{-4}
Vanillin	10.7 ± 2.8	7.0 ± 0.2	6.9 ± 0.5	12.7 ± 3.6	0.373 ± 0.004	0.061 ± 0.002	0.0044 ± 4.1 e^{-4}
Total	104	73.6	50.5	69.6	0.404	0.086	0.0023
Flavonoids							
Luteolin-7-O-G	28.3 ± 3.3	24.3 ± 0.6	23.1 ± 4.7	20.4 ± 3.7	nd	nd	nd
Rutin	50.5 ± 6.3	39.5 ± 1.7	80.2 ± 7.5	33.8 ± 2.3	nd	nd	nd
Apigenin-7-O-G	7.0 ± 1.4	6.2 ± 0.3	7.8 ± 3.3	5.6 ± 1.1	nd	nd	nd
Luteolin	67.1 ± 5.9	60.8 ± 5.2	66.4 ± 1.9	56.0 ± 6.1	0.260 ± 0.001	0.610 ± 0.004	nd
Apigenin	4.0 ± 0.9	3.9 ± 0.2	4.2 ± 0.7	3.5 ± 0.7	0.200 ± 0.002	0.270 ± 0.012	nd
Total	157	135	162	120	0.460	0.880	nd
Lignans							
Acetoxypinoresinol + pinoresinol	nd	nd	nd	nd	21.9 ± 1.6	19.7 ± 2.1	nd

Values are the mean ± SD of four experiments. nd, not detected; tr, traces.

[a] 3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol; 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; *p*-HPEA-EA, aldehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropein aglycone; ME 3,4-DHPEA-EA, methylated form of oleuropein alycone; Luteolin-7-O-G, luteolin-7-*O*-glucoside; Apigenin-7-O-G, apigenin-7-*O*-glucoside.

[b] Not phenolic compound.

and olive oil) resulted in an insignificant amounts of phenolic alcohols in partition conditions.

The highest concentrations of oleuropein and ligstroside and their demethylated structures were detected in olive pastes from the beginning of the production season (Table 1a). There was an significant decrease in these concentrations in the second and, mainly, the third sampling periods, where only demethyloleuropein was quantified (Tables 2a and 3a, respectively). The partition of these compounds only took place between the solid phases and their presence in olive oil has not been reported, reiterating the theory that compounds derived from oleoside are formed during the processing of olive oil. Moreover, the origin of the secoiridoid derivatives in the olive paste could be attributed to the activation of the endogenous β-glycosidase during the crushing operation and its continuous action in the malaxation, as mentioned above [1].

The quantities of secoiridoid derivatives were similar in the oil phases resulting from both horizontal decanting (oil must) and vertical centrifuging (olive oil). However, it appears that the addition of water to the vertical centrifuge implied a slight increase in the 3,4-DHPEA-EDA compound in the virgin olive oil. Such an effect was mainly observed in oils from the beginning of the production season (Table 1a). Moreover, this rise was correlated with the oxidative stability measured by Rancimat. At the first sampling, the oil must had an oxidative stability of 10.1 h, whereas in the extra virgin olive oil, this value reached 11.9 h. At the middle of the season, the oxidative stability was 7.3 and 8.7 h in the oil must and olive oil, respectively. Finally, the oxidative stability measured in the olive must was 5.9 h, compared with 6.6 h for the olive oil at the third sampling.

Free elenolic acid was only found in the oily phases. The concentration of verbascoside, a heterosidic ester of caffeic acid and hydroxytyrosol, did not vary significantly between the olive paste and wet pomace, indicating a significant retention in the solid phases. It should be noted that an opposite relationship between oleuropein and verbascoside content has been reported [24], since the partial degradation of oleuropein is responsible for the formation of verbascoside [1]. However, in this study, it is difficult to establish whether the slightly increased verbascoside content observed during the malaxation step in the first and second samplings (Tables 1a and 2a) corresponded to a decrease in oleuropein.

With regard to the phenolic acids (Tables 1b, 2b and 3b), homovanillic acid was the most abundant compound of this group in the olive pastes, although there was slight decrease in the amount in wet pomaces. In general, a good transference was observed between the olive paste and the wet pomace, and the concentration of this phenolic group decreased throughout the production season, mainly in the solid phases.

In olive paste and wet pomace, the solid phases, the main flavonoids quantified were luteolin followed by rutin. At the same time, low concentrations of the 7-O glycoside forms of luteolin and apigenin were observed, but the transference of the flavonoids was only reflected in the presence of non-glycosides forms in olive must and final virgin olive oil. These results could agree with the information reporting that luteolin has been found not only in pulp but also in olive husks and olive oil [32], whereas rutin has not been detected in olive oil. Lignans (acetoxypinoresinol + pinoresinol) were quantified in both the oil must and the virgin olive oil but were not detectable in the olive paste or wet pomace (solid phases). This could be explained by their lipidic character and by the fact that these compounds could be releasable from the vegetable sources after hydrolysis treatments [33]. The effect of the harvest period on the concentration of flavonoids and lignans was proportionally lower than the effect on the other phenolic fractions.

Molar transfer index

The MTIs were established in order to compare the partitioning of the more important secoiridoid derivative (3,4-DHPEA-EDA) from the olive paste at the end of the malaxation step ($t = 45$ min) to the olive oil (MTIp) as liquid phase, and the partitioning to the wet pomace (MTIw) as solid phase, in the three different periods of the olive oil production season. This allowed the evaluation of the behavior of the main phenolic compound during olive oil process.

The MTIp values of 0.025, 0.057, and 0.089 corresponding to the three samplings (first, middle, and final production periods, respectively) indicated that the transfer of the secoiridoid derivatives from the olive paste to virgin olive oil (lipophilic behavior) increased with the length of the production period, in spite of the lesser secoiridoid content in olive pastes from the final period, which was probably related to the more advanced ripening stage of the olive fruit. Related to this trend, the MTIw values of 1.036, 1.051, and 0.845 corresponding to the first, middle, and final production periods, respectively, indicated that the more important losses of the secoiridoid derivatives with the wet pomace during olive oil extraction process were observed during the early part of the season when the secoiridoid derivatives in olive paste were higher.

Conclusions

In terms of partition, the more important losses of the different phenolic groups present in olive paste occur in the solid phase (wet pomace), and the low lipophilic behavior of the phenolic structures led to a low concentration in virgin olive oil. The transformation of secoiridoid struc-

tures, which took place in the olive paste during the crushing and malaxation operations, led to the formation of secoiridoid derivatives and their detection in the final products of oil must, olive oil, and wastewater. In general, the malaxation step had an important effect on some phenolic groups, mainly on the secoiridoids, and there was a progressive decrease in the total amount of phenolic alcohols as the malaxation time increased. Demethyloleuropein and oleuropein decreased significantly after 45 min of malaxation. This could indicate the beginning of the degradation process of the secoiridoid compounds or their transformation into secoiridoid derivatives, mainly in the 3,4-DHPEA-EDA. However, demethyl-ligstroside and ligstroside showed more stable phenolic structures under the effect of the enzymatic activities during the crushing and malaxation operations.

The differences found between the samplings directly infer that the production season time affects the total phenolic compound content as well as the phenolic profile of olive paste and virgin olive oil. The MTI showed a minor retention of the secoiridoid compounds in wet pomace in the last period of the crop season and a major transference of the secoiridoid derivatives to the final virgin olive oil. This was in spite of the lower phenol content in the olive oil from the end of the production period that corresponds to lower phenol content in olive fruit as a consequence of the advanced ripening process.

Acknowledgements The authors would like to thank the Interministerial Commission on Science and Technology (Ministry of Education and Science, Spain) (Project AGL2002-00289ALI), the Interdepartmental Commission on Research and Technologic Innovation (Catalonian Government) and the European Social Fund for their financial support.

References

1. Ryan D, Antolovich M, Prenzler P, Robards K, Lavee S (2002) Sci Hort 92:147–176. DOI: 10.1016/S0304-4238(01)00287-4
2. Patumi M, d'Andria R, Marsilio V, Fontanazza G, Morelli G, Lanza B (2002) Food Chem 77:27–34. DOI: 10.1016/S0308-8146(01)00317-X
3. Bravo L (1998) Nutr Rev 56:317–333
4. Di Giovacchino L, Sestili S, Di Vincenzo D (2002) Eur J Lipid Sci Technol 104:587–601. DOI: 10.1002/1438-9312(200210)104:9/10<587::AID-EJLT587>3.0.CO;2-M
5. Cerretani L, Bendini A, Rotondi A, Lercker G, Gallina-Toshi T. Eur J Lipid Sci Technol 107:93–100. DOI: 10.1002/ejlt.200401027
6. Gallina-Toschi T, Cerretani L, Bendini A, Bonoli-Carbognin M, Lercker G (2005) J Sep Sci 28:859–870. DOI: 10.1002/jssc.2005000447
7. Morelló JR, Motilva MJ, Tovar MJ, Romero MP (2004) Food Chem 85:357–364. DOI: 10.1016/j.foodchem.2003.07.0128
8. Ranalli A, Gomes T, Delcuratolo D, Contento S, Lucera L (2003) J Agric Food Chem 51:2597–2602. DOI: 10.1021/jf026176x
9. Brenes M, Garcia A, Garcia P, Garrido A (2001) J Agric Food Chem 49:5609–5614. DOI: 10.1021/jf0107860
10. Servili M, Begliomoni A, Montedoro G, Petruccioli M, Federici F (1992) J Sci Food Agric 58:253–260
11. Ranalli A, Serraiocco A (1996) Grasas y Aceites 47:227–236
12. Di Giovacchino L, Ostantini N, Serraiocco A, Surricchio G, Basti C (2001) Eur J Lipid Sci Technol 103:279–285. DOI: 10.1002/1438-9312(200105)103:53.0.CO;2-I
13. Alburquerque JA, Gonzalvez J, Garcia D, Cegarra J (2004) Bioresour Technol 91:195–200. DOI: 10.1016/S0960-8524(03)00177-9
14. Angerosa F, Mostallino R, Basti C, Vito R, Serraiocco A (2000) J Sci Food Agric 80:2190–2195
15. Gimeno E, Castellote AI, Lamuela RM, De la Torre MC, Lopez MC (2002) Food Chem 78:207–211. DOI: 10.1016/S0308-8146(01)00399-5
16. Morelló JR, Vuorela S, Romero MJ, Motilva MJ, Heinonen M (2005) J Agric Food Chem 53:2002–2008. DOI: 10.1021/jf048386a
17. Antolovich M, Bedgood DR Jr, Jardine D, Prenzler PD, Robards K (2004) J Agric Food Chem 52:962–971. DOI: 10.1021/jf0349883
18. Briante R, Patumi M, Terenziani S, Bismuto E, Febbraio F, Nucci R (2002) J Agric Food Chem 50:4934–4940. DOI: 10.1021/jf025540p
19. Carrasco-Pancorbo A, Cerretani L, Bendini A, Segura-Carretero A, Del Carlo M, Gallina-Toshi T, Lercker G, Compagnone D, Fernández-Gutiérrez A (2005) J Agric Food Chem 53:8918–8925. DOI: 10.1021/jf0515680
20. Lesage L, Navarro D, Maunier S, Sigoillot JC, Lorquin J, Delattre M, Simon TL, Asther M, Labat M (2001) Food Chem 75:501–507. DOI: 10.1016/S0308-8146(01)00227-8
21. Artajo LS, Romero MP, Motilva MJ (2006) J Sci Food Chem 86:518–527. DOI: 10.1002/jsfa.2384
22. Artajo LS, Romero MJ, Tovar MJ, Motilva MJ (2006) Eur J Lipid Sci Technol 108:19–27. DOI: 10.1002/ejlt.200500227
23. Spanish Standard UNE 55020 (1973). Instituto Español de Normalización (IRANOR)
24. Amiot MJ, Fleuriet A, Macheix JJ (1986) J Agric Food Chem 34:823–826
25. Gariboldi P, Jommi G, Vrotta L (1986) Phytochem 25:865–869
26. Limiroli R, Consonni R, Ottolina G, Marsilio V, Bianchi G, Zetta L (1995) J Chem Soc 1:1519–1523
27. Brenes M, Rejano L, Garcia P, Sanchez A, Garrido A (1995) J Agric Food Chem 43:2702–2706
28. Servili M, Baldioli M, Selvaggini R, Macchioni A, Montedoro G (1999) J Agric Food Chem 47:12–18. DOI: 10.1021/jf9806210
29. Bianco AD, Muzzalupo I, Piperno A, Romeo G, Uccella N (1999) J Agric Food Chem 47:3531–3534. DOI: 10.1021/jf981240p
30. De Nino A, Mazzotti F, Perri E, Procopio A, Raffaelli A, Sindona G (2000) J Mass Spectrom 35:461–467
31. Rodis PS, Karathanos VT, Mantzavinou A (2002) J Agric Food Chem 50:596–601. DOI: 10.1021/jf010864j
32. Rovellini P, Cortesi N, Fedeli E (1997) Riv Ital Sost Grasse XXIV:273–279
33. Milder IEJ, Arts ICW, Van De Putte B, Venema DP, Hollman PCH (2005) British J Nutr 93:393–402. DOI: 10.1079/BJN20051371