Phenolic Compounds: Their Role During Olive Oil Extraction and in Flaxseed – Transfer and Antioxidant Function

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2.1 TRANSFER OF PHENOLIC COMPOUNDS DURING OLIVE OIL EXTRACTION IN RELATION TO RIPENING STAGE OF THE FRUIT

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Objective 1.1

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Transfer of phenolic compounds during olive oil extraction in relation to ripening stage of the fruit

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Abstract: The transfer of phenolic compounds of *Olea europaea* L. cv. *Arbequina* variety during olive oil extraction in relation to ripening stage was investigated. The parameters of oil extraction by the Abencor system are shown together with mass balances of the products and by products from the olive oil extraction in relation to olive paste. The phenolic compounds in olive paste, pomace, oil and wastewater were identified and measured by HPLC. Throughout the study, the concentrations of simple phenols, secoiridoids and flavonoids were higher in the olive paste and pomace phases than in oil and wastewater phases. High concentrations of 4-(acetoxyethyl)-1,2-dihydroxybenzene (3,4-DHPEA-AC) and secoiridoid derivatives such as the dialdehydic form of elenolic acid linked to 3,4-DHPEA (hydroxytyrosol) or *p*-HPEA (tyrosol) (3,4-DHPEA–EDA, *p*-HPEA–EDA, where EDA is elenolic acid dialdehyde) and an isomer of oleuropein aglycone (3,4-DHPEA–EA, where EA is elenolic acid aldehyde) were found in olive oil, together with lignan compounds. It was observed that 3,4-DHPEA–EDA was the most abundant polyphenol present in the wastewater phase. This indicates that biotransformation occurred during olive extraction, especially in the crushing and malaxation operations, and reflects the possible chemical changes that lead to the formation of new compounds. Moreover, the distribution of compounds showed their affinities toward different phases.

Keywords: polyphenols; olive oil process; olive ripening stage; olive paste; pomace; olive oil; wastewater

INTRODUCTION

Phenolic compounds in food originate from one of the main classes of secondary metabolites in plants derived from phenylalanine and also, to a lesser extent, from tyrosine in some plants.[1] The occurrence of this complex group of substances in plant foods is extremely variable, ranging from simple phenolic molecules to highly polymerized compounds with molecular weights >30 000 Da. Most of these compounds have relative low molecular weights and have variable solubility depending on their polarity and chemical structure, such as degree of hydroxylation, glycosylation or acylation. Some of them, however, can be linked to cell wall components (polysaccharides, lignin). Owing to the nature of the ester linkages, these compounds can be solubilized in alkaline conditions or are otherwise retained in the fiber matrix.[2]

Oleuropein is the major phenolic compound in the pulp of many olive cultivars in which its concentration reaches relatively high levels in immature olive fruit during the growth phase.[2] However, its concentration declines with the physiological development of the fruit in what is termed the green maturation phase and this may be correlated with the increased activity of the hydrolytic enzymes with maturation.[3,4] The level continues to decline rapidly during the black maturation phase characterized by the appearance of anthocyanins.[5]

These changes in drupes are directly reflected in the composition of the olive oil since virgin olive oil is obtained by mechanical or physical methods under conditions, especially temperature, guaranteed to avoid any alteration to the oil. However, many modifications take place in olive compounds as a consequence of cellular destruction and the mixing of cellular content during olive oil extraction (crushing and malaxation). These include hydrolysis of glycerides by lipases, hydrolysis of glycosides and oligosaccharides by glucosidases, oxidation of phenolic compounds by phenol oxidases and polymerization of free phenols.[6] During crushing, secoiridoid aglycones such as 3,4-DHPEA–EDA, *p*-HPEA–EDA and 3,4-DHPEA–EA can be produced by the hydrolysis of oleuropein, demethyloleuropein and ligstroside [throughout, the

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following abbreviations are used: 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene; EDA, dialdehydic form of elenolic acid; 3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol; EA, aldehydic form of elenolic acid]. Besides secoiridoid aglycones, virgin olive oils contain several compounds such as phenolic acids (caffeic, vanillic and *p*-coumaric), phenolic alcohols (3,4-DHPEA and *p*-HPEA), lignans (acetoxypinoresinol and pinoresinol) and flavonoids (luteolin and apigenin).[6]

During the first steps of the oil extraction process, the crushing of the olives in order to break down the cellular membranes and thus release small drops of oil results in an olive paste that is a multiphasic system and the antioxidant partitioning into phases is thermodynamically according to their affinities toward these phases.[7] Moreover, the proportions of antioxidants residing in the three different phases (oil, water and solids) depend on the relative polarities of the antioxidants and the relative amounts of the phases.[7] All operations included in the oil extraction process allow the highest quantity of oil to be obtained from olive fruits and the malaxation step, where small oil droplets formed during milling merge into large drops, is especially useful for achieving high and satisfactory yields of extraction.[8] Several studies have shown that malaxation is the step in oil extraction that especially modifies their qualitative and quantitative composition.[9]

The purpose of this work was to determine the transfer of phenolic compounds during the virgin olive oil extraction process, between the olive paste, pomace, oil and wastewater, in relation to ripening stage of the olive fruits from *Arbequina* cultivar.

MATERIALS AND METHODS

Method for picking olive fruit

The experiment was carried out during the olive harvest period in 2003. Homogeneous batches of 3 kg of olive fruits from the *Arbequina* cultivar, planted on a predominantly clay loam soil located in the Segrià region (Catalonia, Spain), were recollected by hand at three different times from the green stage to black stage in a period of 45 days (November–December). The ripening index (RI) of olive fruit was determined according to the guidelines of the Spanish National Institute of Agronomic Research, based on a evaluation of the olive skin and pulp colours.[10] The procedure consists of distributing a randomly taken sample of 100 fruit into eight groups: intense green (group $N = 0$), yellowish green (group $N = 1$), green with reddish spots (group $N = 2$), reddish brown (group $N = 3$), black with white flesh (group $N = 4$), black with <50% purple flesh (group $N = 5$), black with >50% purple flesh (group $N = 6$) and black with 100% purple flesh (group $N = 7$). The ripening index is given by $\Sigma(in_i)/N$ where i is the number of the group, n_i is the number of olives in it and N is the total number of olives. The ripening indexes used in this study were 2, 5 and 6, which represent different stages of the olive fruit (first, intermediate and black stages, respectively).

Olive oil extraction

The Abencor system (MC2 Ingeniería y Sistemas, Seville, Spain) consists of three essential elements: the mill, the thermobeater and the olive paste centrifuge. The olives were crushed with a hammer mill, the olive paste obtained was malaxated at 28 ± 1 °C for 20 min, then 300 g of water were added at 50 °C and homogenization for 10 min was carried out at 32 ± 1 °C. Finally, centrifugation (1 min, 40 °C, $1445.5 \times g$) was performed, adding 100 g of water, at 50 °C in order to obtain the by-products: pomace, oil and wastewater. The oil was separated from the wastewater by decantation and all oil samples were filtered through a filter-paper of 100 μm porosity (Ahlstrom, Helsinki, Finland). In order to asses mass balance of products and wastes from the Abencor in relation to olive paste, samples with the same ripening index were processed in four lots.

Olive paste and pomace analyses

Moisture content

Samples of 10 g of olive paste and pomace were weighed, then dried for 24 h at 105 °C, cooled for 30 min in a desiccator and reweighed according to the UNE Standard Spanish method (Asociación Española de Normalización y Certificación, Spanish Standard Method UNE 55020, 1973).[11]

Lipid content

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

Both analyses were necessary to obtain a mass balance approach.

Extraction of phenolic compounds

The phenolic extracts of olive paste and pomace were obtained using the method of Tovar *et al.*[12] with modifications. Briefly, 200 g of each sample were crushed with a refrigerated cleaver mill (temperature 4 °C) for 3 min in order to obtain a homogeneous paste. The extraction, purification and separation were done as follows: 4 g of sample were extracted with 80 ml of 80:20 (v/v) ethanol–water containing sodium metabisulfite (400 mg kg^{-1}). The mixture was homogenized using a Polytron homogenizer (Kinematica, Switzerland), centrifuged at $637 \times g$ for 10 min and the supernatant was filtered under vacuum conditions. The pellet was re-extracted as above. The ethanol extract was removed by rotatory evaporation at reduced pressure with a vacuum pump at 31 °C to a volume of 1–2 mL (syrupy consistency). The

purification was carried out with 120 mL of acidified methanol (0.1 mol L^{-1} HCl, pH 2.5) and 40 mL of *n*-hexane were added in order to eliminate the residual oil of the resulting methanolic extract. The separation of the phases was performed by using separating funnels. The purification was done in triplicate. The methanolic extracts were combined (phenolic extract) and rotatory evaporated to dryness under reduced pressure at 31 °C and the residue was dissolved in methanol for HPLC analysis.

Phenols were extracted from olive oil using the method described by Morelló *et al*.[13] Methanol–water (80:20 v/v) (2 × 20 mL) was added to 45 g of virgin olive oil and homogenized for 2 min with a Polytron. The two phases were separated by centrifugation at 637 × *g* for 10 min. The extracts were combined and concentrated under vacuum at 31 °C until a syrupy consistency was reached. The phenol extract was dissolved in 5 mL of acetonitrile and washed with 3 × 20 mL of *n*-hexane. The apolar phases were treated with 5 mL of acetonitrile. The acetonitrile solution was then rotatory evaporated to dryness under vacuum and the residue was dissolved in 5 mL of acetonitrile. Finally, an aliquot of 2 mL was evaporated under a nitrogen stream.

The wastewater was separated from oil by decanting and then filtered through a filter-paper (10 µm) under vacuum. The extract obtained was filtered through a 0.45 µm filter and injected into the chromatograph as described by Romero *et al*.[14]

HPLC analysis of phenolic compounds

The extracted phenolic fractions were dissolved in 1 mL of methanol and analyzed by HPLC (injection 20 µL). The HPLC system included a Waters 600 E pump, a Waters column heater (column temperature 22 °C), a Waters 717 Plus autosampler and a Waters 996 photodiode-array detector (Waters,

Table 1. Total mass balance during the olive oil extraction process[a]

	Input (g)				Output (g)			
RI	Olive paste	Malaxing water	Centrifugation water	Total	Oil	Wastewater	Pomace	Total
2	801.93	301.32	105.97	1209.21	99.51	363.33	718.61	1181.45
5	700.00	305.00	100.00	1105.00	102.48	332.18	636.84	1071.49
6	712.97	302.62	103.59	1119.19	154.41	215.42	706.61	1076.44
	Input (%)				Output (%)			
2	73.76	24.92	8.76	100.00	8.23	30.05	59.43	97.71
5	63.34	27.60	9.05	100.00	9.27	30.06	57.63	96.96
6	63.70	27.04	9.26	100.00	13.79	19.26	63.12	96.18

[a] Values represent the means from four experiments. RI, ripening index.

Table 2. Component balance during the olive oil extraction process[a]

	Input (g)				Output (g)			
RI	Olive paste	Malaxing water	Centrifugation water	Total	Oil	Wastewater	Pomace	Total
Water balance in process								
2	404.70	301.32	105.97	811.99	0.00	334.27	461.22	795.49
5	308.95	305.00	100.00	713.95	0.00	272.15	415.16	687.31
6	292.04	302.62	103.59	594.66	0.00	188.77	449.05	637.82
	Input (%)				Output (%)			
2	49.84	37.11	13.05	100.00	0.00	41.18	56.80	97.98
5	43.76	41.86	14.38	100.00	0.00	38.14	58.13	96.27
5	41.87	43.27	14.88	100.00	0.00	27.05	64.30	91.35
Oil balance in process								
	Input (g)				Output (g)			
2	207.42	0.00	0.00	207.42	99.51	0.00	86.87	186.38
5	193.37	0.00	0.00	193.37	118.95	0.00	66.41	185.36
6	232.86	0.00	0.00	232.86	154.41	0.00	75.15	229.55
	Input (%)				Output (%)			
2	100.00	0.00	0.00	100.00	48.03	0.00	42.01	90.04
5	100.00	0.00	0.00	100.00	61.92	0.00	34.23	96.15
6	100.00	0.00	0.00	100.00	66.40	0.00	32.30	98.69

[a] Values represent the means from four experiments. RI, ripening index.

Milford, MA, USA). The column was an Inertsil ODS-3 (5 µm, 15 cm × 4.6 mm i.d.) (GL Sciences, Tokyo, Japan) equipped with a Spherisorb S5 ODS-2 precolumn (5 µm, 1 cm × .6 mm i.d.) (Technokroma, Barcelona, Spain). Empower software was used and chromatograms were obtained at 278 and 339 nm.

Reference compounds

Oleuropein, verbascoside, apigenin, luteolin, tyrosol and *p*-coumaric acid were obtained from Extrasynthèse (Genay, France) and vanillic acid and vanillin from Fluka (Buchs, Switzerland). Hydroxytyrosol was donated by Professor Montedoro (University of Perugia, Italy). The rest of the phenolic compounds were isolated from olive oil extract samples using a Spherisorb ODS-2 semi-preparative HPLC column (5 µm, 25 cm × 10 mm i.d. (Technokroma) and a flow-rate of 4 mL min^{-1}. The mobile phases and gradient have been described elsewhere.[13] Individual phenols were quantified by a four-point regression curve on the basis of standards obtained from commercial suppliers or from semi- preparative HPLC as described above. Quantification of the phenolic compounds was carried out at 278 and 339 nm. Individual phenols of olive paste, pomace, olive oil and wastewater are expressed as mg kg^{-1}.

RESULTS AND DISCUSSION

Mass and component balance during the oil extraction process

During the olive oil extraction process, the temperature and malaxation time were considered constants in order to asses the real influence of the ripening index of olive fruit on phenol transfer between the different phases. The olive fruits were crushed and destruction of the tissue structure occurred, then the olive paste (input solid phase) obtained was malaxed to make a continuous oily phase. Finally, centrifugation allowed the oil contained in the paste to be separated.

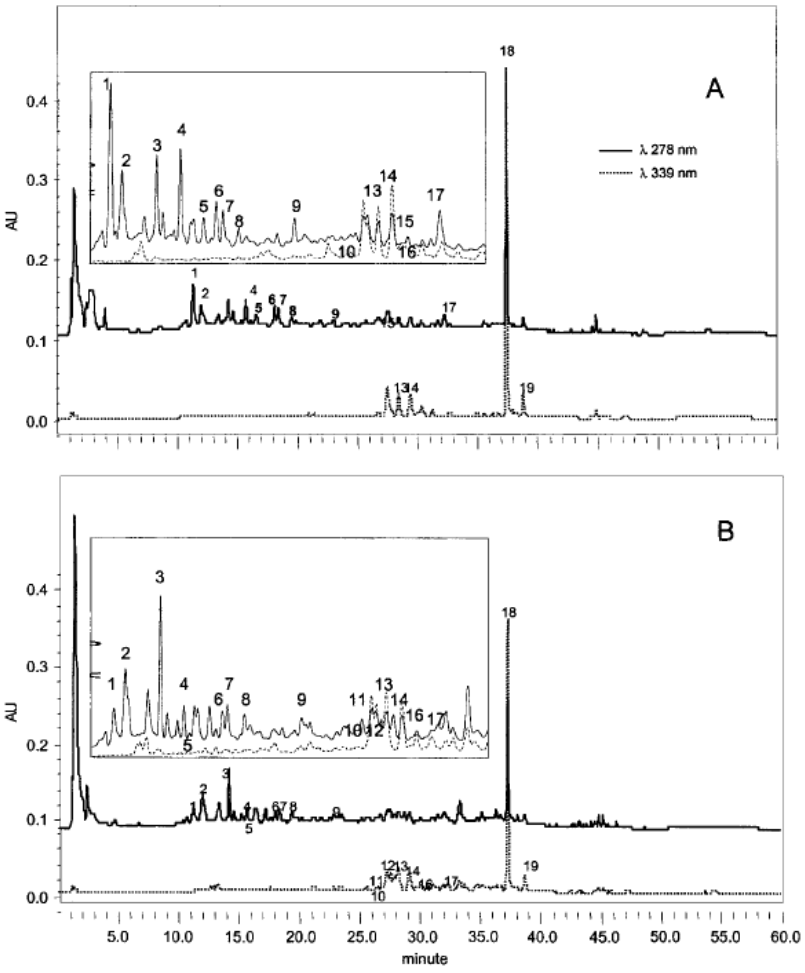

Figure 1. Chromatographic profiles of the phenolic compounds of the solid phases. (A) Olive paste; (B) pomace. See Table 3 for identification of peaks.

The system has two inputs, olive paste and water, and two outputs with three by-products: pomace and wastewater–oil (Table 1).

In the experimental process, olive paste (raw material) is processed with water in order to obtain a complex matrix system and the input of water in the process is around 32% of the total material input. From the results shown in Table 1, it is clear that among the by-products, pomace represents the majority of the products obtained, whereas oil does not reach 14% in all three samples. The higher the ripening index of olive fruit, the greater is the oil yield in the process. Water and oil balances were performed to observe the distribution of each component in all the by-products. The moisture and lipid contents were calculated in every case. Thus, with previous calculations, the data shown in Table 2 refer to the amounts of water and oil present in the olive paste and during the process. Table 2 also reveals a decrease in moisture content in olive paste with increase in ripening index. Direct comparison of the by-products shows an increase in the percentage of water in the pomace phase with olive ripening.

Phenolic profile of the solid and liquid phases

The chromatographic profiles of the phenolic compounds of the solid phases, olive paste and pomace and flavonoids from the *Arbequina* cultivar are showed in Fig. 1 and the identified phenolic components, retention times and average concentrations of the solid phases are shown in Table 3. The olive paste compounds include simple phenols, phenolic acids, aldehydes, secoiridoid glycosides and flavonoids. Peak 4 could be considered simple phenols based on their retention time and spectral characteristics. This would imply that the derived products formed with olive paste are probably related to the crushing operation, which allows the biotransformation of hydroxytyrosol. Moreover, peak 17 presents similar spectral characteristics to those of oleuropein and it could be a derivative

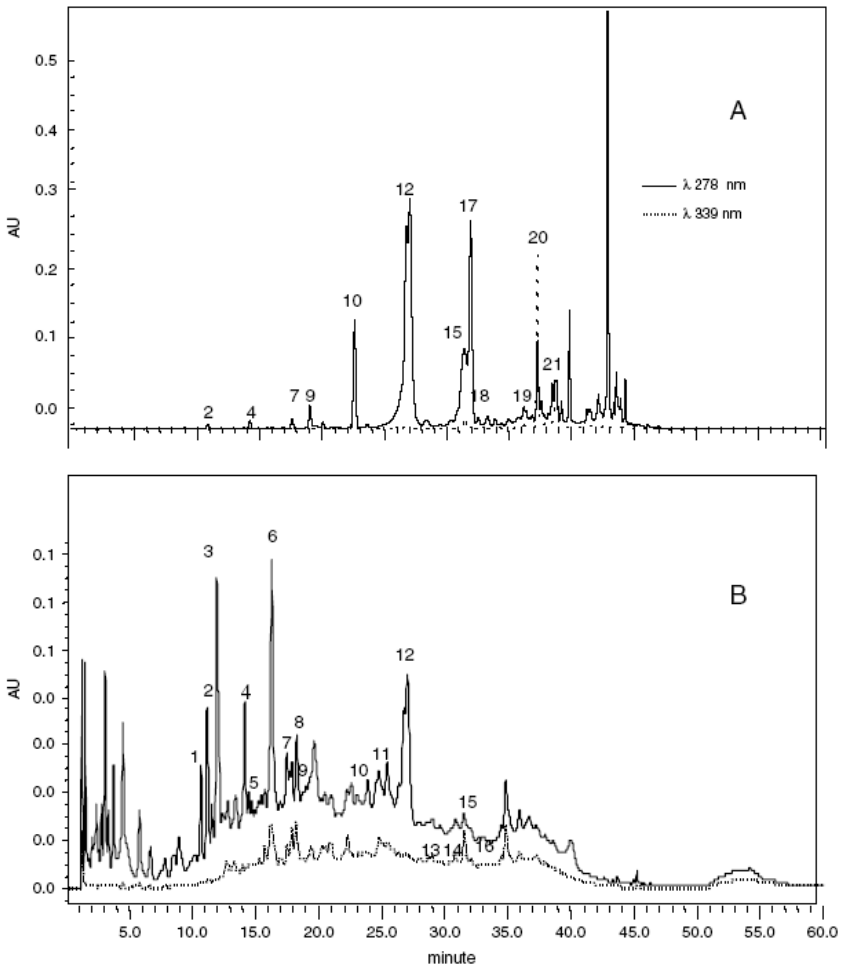

Figure 2. Chromatographic profiles of the phenolic compounds of the liquid phases. (A) Olive oil; (B) wastewater. See Table 4 for identification of peaks.

Table 3. Identification, retention time (RT) and concentration of polyphenolic compounds in the solid phases, olive paste and pomace from olive oil extraction process (cv. *Arbequina*)

			Concentration (mg kg^{-1})			
			Olive paste		Pomace	
Peak No.	RT (min)	Phenolic compound[a]	Mean[b]	Range[c]	Mean[b]	Range[c]
		Simple phenols				
1	11.2	3,4-DHPEA	125.39	93.77–161.04	68.92	58.82–78.38
2	12.0	3,4-DHPEA species	106.40	81.94–134.90	46.91	36.36–53.96
3	14.2	*p*-HPEA	86.49	82.84–92.59	77.74	45.66–110.45
4	15.9	Peak 4	51.14	45.74–59.42	488.47	257.01–653.25
5	16.0	Peak 5	tr[d]		tr	
		Phenolic acids				
6	17.9	Vanillic acid	15.37	12.58–18.29	7.55	5.04–9.04
7	18.4	Homovanillic acid	31.85	31.00–32.60	17.88	13.66–20.93
		Aldehydes				
8	19.4	Vanillin	17.62	13.50–25.33	28.39	25.89–32.73
		Secoiridoid				
9	22.9	Dimethyloleuropein	tr		tr	
10	25.0	*Verbascoside*	tr		tr	
		Flavonoids				
11	27.4	Peak 11	ND[e]		tr	
12	27.7	Peak 12	ND		tr	
13	28.3	Luteolin-7-glucoside	50.65	26.85–63.86	30.06	25.33–36.73
14	29.1	Rutin	84.87	41.52–114.80	52.69	32.57–67.48
		Secoiridoid				
15	29.6	Oleuropein	38.06	33.25–43.37	ND	
		Flavonoid				
16	30.1	Apigenin-7-glucoside	7.06	6.81–7.52	4.00	2.86–5.10
		Secoiridoid				
17	32.2	Peak 17	68.92	54.59–92.58	tr	
		Flavonoids				
18	37.3	Luteolin	113.11	96.51–136.39	91.87	70.64–115.79
19	38.7	Apigenin	5.18	4.65–6.09	3.07	2.33–3.91

[a] 3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol.
[b] Values represent the means from three experiments.
[c] Range of concentration varies from the first to the third sampling.
[d] tr = trace amount.
[e] ND = not detected.

secoiridoid compound formed during the crushing operation as a result of the activation of the endogenous β-glucosidases.[3] Flavonoids constitute the rest of the polyphenols quantified. Phenolic derivatives of secoiridoid compounds were not identified in the olive paste, as observed by Ryan *et al*.[3]

The chromatographic profiles of the phenolic compounds of the liquid phases, olive oil and wastewater are shown in Fig. 2 and the identified phenolic compounds, retention times and average concentrations of the liquid phases are shown in Table 4. The phenolic compounds found in olive oil were in accordance with those reported in other studies of the *Arbequina* cultivar by our group.[15] It was observed that virgin olive oil shows low concentrations of simple phenols and phenolic acids and large amounts of secoiridoid derivatives, such as 3,4-DHPEA–EDA, 3,4-DHPEA–EA and *p*-HPEA–EDA (Table 4). This is in agreement with the results reported by Servili *et al*.[16] and may reflect a chemical change during the oil extraction process, where a mechanical operation leads to the formation of derivatives of the phenolic compounds that occur in olive fruit.

The wastewater phase is a complex matrix system, where a number of secoiridoid derivatives were observed (Table 4). These identification results have been confirmed in several studies.[17–19] Moreover, it should be noted that Romero *et al*.[14] reported a very polar phenolic compound, 4-β-D-glucoside, which appears before hydroxytyrosol, for the first time. In our study, the compound corresponding to peak 1 was found only in the wastewater phase, hence it seems likely to be a hydroxytyrosol species with higher affinity for the aqueous phase. Moreover, the compound called a hydroxytyrosol species was found in wastewater and not in oil and a different polar affinity could reflect its behavior.

The presence of all derivative compounds mentioned (Table 4) could be supported by the fact that the degradative pathways of the phenolic oleosides (oleuropein) would lead to the formation of derivative

Table 4. Identification, retention time (RT) and concentration of polyphenolic compounds in the liquid phases, olive oil and wastewater from olive oil extraction process (cv. *Arbequina*)

			Concentration (mg kg^{-1})			
			Olive oil		Wastewater	
Peak No.	RT (min)	Phenolic compound[a]	Mean[b]	Range[c]	Mean[b]	Range[c]
		Simple phenols				
1	10.6	Peak 1	ND[d]		10.86	6.63–13.71
2	11.0	3,4-DHPEA	0.11	0.07–0.13	22.00	14.35–21.37
3	11.8	3,4-DHPEA specie	ND		37.86	31.15–44.56
4	14.1	*p*-HPEA	0.24	0.16–0.36	29.63	19.78–38.91
5	15.6	Peak 5	ND		tr[e]	
6	16.2	Peak 6	ND		tr	
		Phenolic acids				
7	17.8	Vanillic acid	0.08	0.07–0.09	1.68	1.68
8	18.3	Homovanillic acid	ND		11.79	7.65–15.94
		Aldehydes				
9	19.9	Vanillin	0.08	0.07–0.09	tr	
10	22.6	3,4-DHPEA-AC	17.24	16.48–18.73	tr	
11	22.9	*p*-Coumaric acid			tr	
		Secoiridiod derivative				
12	26.6	3,4-DHPEA–EDA	131.77	78.08–163.41	406.48	230.67–590.25
		Flavonoids				
13	28.7	Luteolin 7-glucoside	ND		8.25	5.89–10.61
14	30.0	Apigenin 7-glucoside	ND		1.25	0.99–1.06
		Secoiridoid derivative				
15	30.9	*p*-HPEA–EDA	11.31	7.84–15.66	tr	
16	31.8	Verbascoside	ND		5.61	4.48–6.73
17	31.9	Lignans	13.34	11.78–16.24	ND	
		Secoiridoid derivatives				
18	32.5	*p*-HPEA–EA	0.61	0.59–0.65	ND	
19	36.1	3,4-DHPEA–EA	12.31	8.91–17.12	ND	
		Flavonoids				
20	37.1	Luteolin	0.94	0.49–1.23	ND	
21	38.6	Apigenin	0.29	0.25–0.32	ND	

[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.
[b] Values represent the means from three experiments.
[c] Range of concentration varies from the first to the third sampling.
[d] ND = not detected.
[e] tr = trace amount.

products especially during crushing and malaxing operations. Taking into account that the aglycone formed by the action of β-glucosidases is degraded in aqueous solution in order to form dialdehydes, the addition of malaxing water and centrifugation water could allow the formation of these derivatives.[20] The high levels of 3,4-DHPEA–EDA observed throughout the study indicate its significance in the overall oil extraction products and by-products, especially in the wastewater phase.

Effect of the olive ripening index on phenol transfer during oil extraction process

The transfer of polyphenols from olive paste to pomace (solid phase) during the oil extraction process in relation to the ripening index of the olive fruit is shown in Table 5. It should be noted that the simple phenols constitute an important group to be analyzed. The hydroxytyrosol concentration in olive paste varies during ripening without a clear trend, as in pomace. However, the transfer percentage to pomace is 45%, being higher in the second sampling. An increase in hydroxytyrosol species (peak 2) was found in paste with an increase in ripening index, although the transfer to pomace decreased only slightly. Despite the high tyrosol concentration in pomace in the second sampling, the concentration of this compound remained relatively constant in all olive paste samples, transferring around 48% at the final ripening index. As can be seen, the concentration of the phenol in olive paste corresponding to peak 4 (Fig. 1) was the lowest relative to the peaks of the rest of the simple phenols in that phase. However, this peak had a very high concentration in pomace, increasing with ripening index. In fact, it is possible to suggest a biotransformation rather than a compound transfer.

Table 5. Distribution of common polyphenols in olive paste and pomace (solid phases) during the oil extraction process in relation to the ripening index of the olive fruit

	Ripening index (RI)[b,c]					
	2		5		6	
Phenolic compound (mg kg^{-1})[a]	Olive paste	Pomace	Olive paste	Pomace	Olive paste	Pomace
3,4-DHPEA	121.36	58.82	93.77	69.57	180.92	78.38
3,4-DHPEA species (peak 2)	81.94	36.36	102.36	50.43	151.55	53.96
p-HPEA	92.59	77.10	82.84	110.45	94.41	45.66
Peak 4	59.42	257.01	48.26	653.25	51.39	555.15
Peak 5	tr[d]	tr	tr	tr	tr	tr
Vanillic acid	18.29	8.56	12.58	5.04	17.12	9.04
Homovanillic acid	31.00	19.05	31.95	13.66	36.62	20.93
Vanillin	25.33	26.56	14.02	25.89	15.16	32.73
Demethyloleuropein	tr	tr	tr	tr	tr	tr
Verbascoside	tr	tr	tr	tr	tr	tr
Luteolin 7-glucoside	26.85	25.33	63.86	28.13	68.82	36.73
Rutin	41.52	32.57	98.28	58.01	128.97	67.48
Oleuropein	130.13	tr	33.25	tr	115.68	tr
Apigenin 7-glucoside	6.81	5.10	6.86	4.05	8.45	2.86
Peak 17	92.58	ND	54.59	ND	59.6	ND[e]
Luteolin	96.51	70.64	106.43	89.18	153.22	115.79
Apigenin	6.09	3.91	4.80	2.99	5.22	2.33
Total	830.44	621.01	753.84	1108.81	1087.15	1022.90

[a] 3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol.
[b] Ripening index: 2, skin green with reddish spots; 5, skin black with <50% purple flesh; 6, skin black with >50% purple flesh.
[c] Values are means of eight determinations.
[d] tr = trace amount.
[e] ND = not detected.

As the fruits were very ripe, crushing and malaxation may have caused the rupture of intracellular tissues, leading to the formation of new compounds.

In relation to phenolic acids, the percentage transfer of vanillic and homovanillic acids from paste to pomace was similar at all ripening indexes. Vanillin decreased with increase in ripening index in the olive paste, although a high transfer was observed with the values reached at the third sampling. Among flavonoids, the luteolin 7-glucoside concentration in the olive paste was low at the first sampling in relation to the second and third sampling, where it remained high. Meanwhile, the highest transfer to pomace was identified at the second ripening index. The rutin concentration increased in the paste with increase in the ripening stage whereas, in contrast, the transfer to pomace decreased until it reached 52%.

Oleuropein, the most important secoiridoid and the precursor of important antioxidant compounds occurring in oil, was only detected at low concentrations in olive paste, as reported by Morelló *et al.*[21] for the *Arbequina* variety in similar date samplings. In the same way, demethyloleuropein was detected but its concentration was not quantified. Apparently, this could imply no formation of derivative products in oil. However, the peak with a 32.2 min retention time (peak 17) had similar characteristics to the secoiridoid compounds and could explain the presence of derivative products in oil. This peak was only found in paste and its concentration was higher at the first sampling than the second and third samplings. Apigenin 7-glucoside shows an increase in the olive paste in proportion with the increase in the ripening index. However, a decrease was observed in the pomace throughout the study. Luteolin showed a clear increasing trend in all the samplings. In contrast, its concentration reflects a constant transfer from olive paste to pomace from the first to the third sampling. The amounts of apigenin in the paste phase are similar when compared with those of its non-glycosylated form; a decrease in the percentage transfer through the study was also observed.

The transfer of the phenolic compounds between liquid phases showed that the hydroxytyrosol concentration increased slightly in the oil phase in addition to its transfer in relation to olive paste (raw material) with ripening (Table 6). The partition to the wastewater phase was high in the second sampling. The behavior of tyrosol was similar to that of hydroxytyrosol in oil and wastewater, indicating the affinity of simple phenols for liquid phases, being low compared with that in solid phases. Vanillic acid remained constant in oil during all analyses and its transfer to liquid phases reached a maximum in wastewater in the last sampling. However, homovanillic acid was not found in oil. The compound 3,4-DHPEA-AC and secoiridoid derivatives, such as 3,4-DHPEA–EDA, *p*-HPEA–EDA, HPEA–EA and 3,4-DHPEA–EA, whose main precursors are oleuropein and dimethyloleuropein, were identified and quantified in the oil

Table 6. Distribution of common polyphenols in olive oil and wastewater (liquid phases) during the oil extraction process in relation to the ripening index of the olive fruit

	Ripening index (RI)[b,c]					
	2		5		6	
Phenolic compound (mg kg^{-1})[a]	Oil	Wastewater	Oil	Wastewater	Oil	Wastewater
3,4 DHPEA species (peak 1)	ND[d]	13.71	ND	12.23	ND	6.63
3,4-DHPEA	0.07	21.37	0.12	30.29	0.13	14.35
3,4-DHPEA species (peak 3)	ND	31.15	ND	44.56	ND	37.15
p-HPEA	0.16	19.78	0.21	38.91	0.36	30.20
Vanillic acid	0.07	1.68	0.07	tr[e]	0.09	tr
Homovanillic acid	ND	7.65	ND	15.94	N.D.	8.5
Vanillin	0.07	tr	0.09	tr	0.09	tr
3,4-DHPEA-AC	16.52	tr	16.48	tr	18.73	tr
p-Coumaric acid	ND	tr	ND	tr	ND	tr
3,4-DHPEA–EDA	78.08	230.67	153.81	582.30	163.41	590.25
p-HPEA–EDA	7.84	tr	10.44	tr	15.66	tr
Lignans	12.00	ND	11.78	ND	16.24	ND
p-HPEA–EA	0.60	ND	0.59	ND	0.65	ND
3,4-DHPEA–EA	10.92	ND	8.91	ND	17.12	ND
Luteolin 7-glucoside	ND	ND	ND	10.61	ND	5,89
Apigenin 7-glucoside	ND	1.06	ND	1.70	ND	0,99
Verbascoside	ND	4.48	ND	6.73	ND	tr
Luteolin	0.49	ND	1.11	ND	1.23	ND
Apigenin	0.25	ND	0.29	ND	0.32	ND
Total	127.19	318.31	204.04	731.48	234.24	187.54

[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.
[b] Ripening index: 2, skin green with reddish spots; 5, skin black with <50% purple flesh; 6, skin black with >50% purple flesh.
[c] Values are means of eight determinations.
[d] ND = not detected.
[e] tr = trace amount.

phase. However, it should be noted that the affinity of 3,4-DHPEA–EDA with wastewater was really significant.

Among the group of glycosylated flavonoids, luteolin 7-glucoside and apigenin 7-glucoside were not identified in oil at any ripening stage. Moreover, their transfer to wastewater was low. Verbascoside was only quantified in the wastewater phase, although its concentration showed an undetermined trend at all three ripening indexes. Finally, luteolin and apigenin reflected a slight increase in oil with increase in the ripening stage of the olive fruit and they were not transferred to wastewater.

CONCLUSIONS

It can be stated that the olive oil extraction process results in molecular biotransformations and chemical reactions in the phenolic compounds.

The simple phenols constitute an important group to study owing to their transfer and transformation through the olive process being especially notable in solid phases. In general, an increase in the ripening index implies an increase in the hydroxytyrosol concentration found in pomace. The flavonoids luteolin-7-glucoside, rutin and luteolin show a clear trend to increase with the increase in ripening index in all phases evaluated (olive paste, pomace, oil and wastewater). Finally, the phenolic compound 3,4-DHPEA-AC and the secoiridoid derivatives 3,4-DHPEA–EDA, *p*-HPEA–EDA, *p*-HPEA–EA and 3,4-DHPEA–EA, whose main precursors are oleuropein and demethyloleuropein, are present in the oil phase. It should be noted that the affinity of 3,4-DHPEA–EDA with wastewater was very significant.

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REFERENCES

1 Shahidi F and Naczk M, Phenolics, in *Food and Nutraceuticals*. CRC Press LLC, Boca Raton, FL, pp 1–16 (2004).
2 Bravo L, Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. *Nutr Rev* 56:317–333 (1998).
3 Ryan D, Antolovich M, Prenzler P, Robards K and Lavee S, Biotransformations of phenolic compounds. *Sci Hort* 92:147–176 (2002).
4 Amiot MJ, Fleuriet A and Macheix JJ, Accumulation of oleuropein derivatives during maturation. *Phytochemistry* 28:67–69 (1989).

5 Limiroli R, Consonni R, Ottolina G, Marsilio V, Bianci G and Zetta L, ^{1}H and ^{13}C NRM characterization of new oleuropein aglycones. *J Chem Soc, Perkin Trans 1* 1519–1523 (1989).
6 Ryan D and Robards K, Phenolic compounds in olives. *Analyst* 123:31R–44R (1998).
7 Rodis PS, Karathanos VJ and Mantzavinou A, Partitioning of olive oil antioxidants between oil and water phases. *J Agric Food Chem* 50:596–601 (2002).
8 Angerosa F, Mostallino R, Basti C and Vito R, Influence of malaxation temperature and time on the quality of virgin olive oils. *Food Chem* 72:19–28 (2001).
9 Montedoro G, Baldioli M and Servili M, Sensory and nutritional relevance of phenolic compounds in olive oil. *G Ital Nutriz Clin Prevent* 1:19–32 (1992).
10 Hermoso M, Uceda M, García A, Morales B, Frías ML and Fernández A, *Elaboración de Aceite de Calidad (Serie Apuntes 5/92)*. Consejería de Agricultura y pesca, Seville (1991).
11 Asociación Española de Normalización y Certificación, *Spanish Standard UNE 55020* (1973).
12 Tovar MJ, Romero MP, Girona J and Motilva MJ, L-Phenylalanine ammonia-lyase activity and concentration of phenolics in developing olive (*Olea europaea* L. cv. *Arbequina*) fruit grown under different irrigation regimes. *J Sci Food Agric* 82:892–898 (2002).
13 Morelló JR, Motilva MJ, Tovar MJ and Romero MP, Changes in commercial olive oil (cv. *Arbequina*) during storage, with special emphasis on the phenolic fraction. *Food Chem* 85:357–364 (2004).
14 Romero C, Brenes M, García P and Garrido A, Hydroxytyrosol 4-β-D-glucoside, an important phenolic compound in olive fruits and derived products. *J Agric Food Chem* 50:3835–3839 (2002).
15 Tovar MJ, Motilva MJ and Romero MP, Changes in the phenolic composition of virgin olive oil form young trees (*Olea europaea* L. cv. *Arbequina*) grown under linear irrigation strategies. *J Agric Food Chem* 49:5502–5508 (2001).
16 Servili M, Baldioli M, Selvaggini R, Miniati E, Macchioni A and Montedero F, High-performance liquid chromatography evaluation of phenols in olive fruit, virgin olive oil, vegetation waters and pomace and 1D- and 2D-nuclear magnetic resonance characterization. *J Am Oil Chem Soc* 76:873–882 (1999).
17 Vierhuis E, Servili M, Baldioli M, Schols HA, Voragen AGJ and Montedoro G, Effect of enzyme treatment during mechanical extraction of olive oil on phenolic compounds and polysaccharides. *J Agric Food Chem* 49:1218–1223 (2001).
18 Brenes M, García A, García P, Rios JJ and Garrido A, Phenolic compounds in Spanish olive oil. *J Agric Food Chem* 47:3535–3540 (1999).
19 Montedoro G, Servili M, Baldioli M, Selvaggini R and Miniati E, Simple and hydrolyzable compounds in virgin olive oil. III. Spectroscopic characterization of the secoiridoid derivatives. *J Agric Food Chem* 41:2228–2234 (1993).
20 Bianco A, Buiarelli F, Cartoni G, Coccioli F, Jasionowska R and Margherita P, Analysis by liquid chromatography–tandem mass spectrometry of biophenolic compounds in olives and vegetation waters. Part I. *J Sep Sci* 26:409–416 (2003).
21 Morelló JR, Romero MP and Motilva MJ, Effect of the maturation process of the olive fruit on the phenolic fraction of drupes and oils from Arbequina, Farga and Morrut cultivars. *J Agric Food Chem* 52:6002–6009 (2004).