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

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2.2 EFFECT OF IRRIGATION APPLIED TO OLIVE TREES (*Olea europaea* L.) ON PHENOLIC COMPOUND TRANSFER DURING OLIVE OIL EXTRACTION

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Objective 1.2

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Effect of irrigation applied to olive trees (*Olea europaea* L.) on phenolic compound transfer during olive oil extraction

The main objective of this research was to determine the extent to which irrigation practices affect the partitioning of phenolic compounds between olive paste, pomace, olive oil and wastewater. The current paper also aimed to study the effect of technological natural micro-talc (NMT) addition during the oil extraction process on the partitioning of the phenolic compounds between solid and liquid phases. The results obtained in this study showed that irrigation applied to olive trees let to a considerable decrease in the phenol content of the olive paste. The water status of the 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. The most remarkable point of the phenol partitioning was related to the simple phenols. While in the samples from non-irrigated trees the greater proportion of these phenols partitioned into the pomace, in samples from irrigated trees most of them were lost in the wastewater. After comparison of the results obtained from the experiments with and without NMT addition, it was concluded that the use of that co-adjuvant did not significantly alter either the phenolic profile of the oil phase obtained or the content of the individual phenolic compounds.

Keywords: Irrigation, virgin olive oil, phenol transfer, olive oil extraction, natural micro-talc.

1 Introduction

The scientific and commercial interest in food phenolics has greatly increased as a result of reports about the antioxidant and free-radical-scavenging abilities of some of these compounds and their potential effects on human health, contributing to the prevention of degenerative processes. Furthermore, there has been renewed interest in natural sources of antioxidants, which generally belong to the phenolic group of compounds.

It is widely known that the composition of the phenolic fraction of oils depends on the cultivar, the climatic conditions during growth, the degree of maturation and the agronomic practices related to irrigation treatment [1, 2]. Different studies have demonstrated the influence of these factors, as well as of the production and extraction technologies, on the quality of olive oil in terms of oxidative stability and sensory analysis. A higher quality of the olive oil was obtained at the initial ripeness stage due to its composition in phenolic compounds. At the same time, a decrease in positive sensorial parameters of olive oil with increasing ripening degree was observed [3, 4]. Industrial production and low-scale processing of olive oil determined a different phenolic profile of the resulting product. In general, the content of *o*-diphenols and the oxidative resistance were higher in oils obtained by the low-scale mill process [5, 6].

Moreover, prior studies carried out by our research group proved that different irrigation strategies applied to olive trees (Arbequina cv.) – linear irrigation strategy and regulated deficit irrigation strategy – not only affected the total amount of phenolic compounds present in virgin olive oil but also their HPLC profiles [7, 8]. Different hypotheses were developed to explain the differences in the phenol content of oils from olive trees under irrigation. Water availability, considered as water stress, could influence phenolic metabolism and increase the synthesis of phenolic compounds in the fruit, and therefore in the oil obtained from these. This theory was supported by the results obtained by Patumi et al. [9] and our research group [10] which showed that the phenolic content of the oils depended on the L-phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity in the fruit, which varied with changes in water status.

Another factor that could explain the differences in the oil phenol content is the higher water content of the olives from the trees under irrigation. As phenolic compounds are soluble in both water and oil, significant amounts of these constituents are carried away from the oily phase

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during the process of oil extraction because of partitioning between two non-mixable liquids. Consequently, the phenolic content is closely dependent on the extraction process as this determines the partitioning behavior of the phenolic compounds and hence their distribution between the oil and waste fractions [11].

During crushing and malaxation, steps that break cell walls and expose the matrix to enzymes, oxygen and mild heat, many chemical and enzymatic processes take place. Phenolic compounds are reactive chemical species, vulnerable to oxidation, conjugation, hydrolysis, polymerization and complexation. This is compounded by direct contact with enzymes and their substrates as the cells are no longer intact [12]. In addition, the lipoprotein membranes that surround the oil droplets are removed and re-formed, resulting in a mutual exchange of components between the oil and the water phase. On the other hand, such membranes bind the minute oil droplets to the water droplets and the vegetable colloids, forming stable emulsions, which cannot be isolated or removed by mechanical means. The emulsion is carried away with the by-products, pomace and vegetation water [13].

Some olive varieties and, in general, only olive fruits slightly ripe give rise to the so-called "difficult olive paste" during the olive oil extraction process. In such cases, part of the oil is lost with the residues, lowering the oil yield. In order to break the emulsion, mixing time or temperature could be increased, leading to a loss of oil quality. In order to avoid this problem, the addition of micronized talc (hydrated magnesium silicate of particle size lower than 40 μm) was introduced in the late 1970s. This adjuvant helps to yield oil with lower water and to suspend solid contents, as well as residues with a lower fat content [14]. Moreover, it is eliminated during centrifugation together with the solid residue.

The main objective of this research was to determine the extent to which irrigation practices affect the partitioning of phenolic compounds between olive paste, pomace, olive oil and wastewater. Furthermore, the current paper aimed to study the effect of technological natural microtalc (NMT) addition during the oil extraction process on the partitioning of phenolic compounds between the solid and liquid phases.

2 Materials and methods

2.1 Samples

The experiment was carried out during the olive harvest period (November, 2003) in the Segrià district in the producing area of "Les Garrigues" (Catalonia, Spain). Homogeneous batches of 3 kg of olive fruit from the Arbequina variety grown under non-irrigation (control) and irrigation treatment were hand-picked at a similar ripening index (green with reddish spots). The experimental irrigation implementation was based on a linear irrigation design in which the total applied irrigation water changed linearly with the effective crop coefficient (K_c) used when the water budget method, proposed by the FAO [15], was applied to determine the crop water requirements (ET_c). This used the reference crop evapotranspiration (ET_o) from an agronomic weather station and the effective crop coefficient (K_c) ($Et_c = ET_o \times K_c$). The water budget method calculates the irrigation requirements by subtracting the effective precipitation (P_{ef}) from the ET_c. The irrigation treatment applied had a $K_c = 0.85$, corresponding approximately to an annual water application of 260 mm.

2.2 Ripening index

The olive ripening index was determined according to the proposals of the Spanish National Institute of Agronomic Research [16], based on the evaluation of the olive skin and pulp colors.

2.3 Olive oil extraction process

The Abencor system (MC2 Engineering and Systems, Seville, Spain), a pilot plant scale installation, was used to process the olives. The extraction process consisted of the following: The olives were crushed with a hammer mill, the olive paste obtained was malaxated at 28 ± 1 °C for 20 min; then, 300 g water at 50 °C was added and the mixture homogenized for 10 min. Before centrifugation, another 100 g of water at 50 °C was added 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 paper filter.

To study the effect of the addition of a technological coadjuvant in the process of oil extraction, the samples from the non-irrigation treatment (control) were malaxated with and without addition of NMT (10 g). All the samples were processed in two batches.

2.4 Phenolic extraction

The phenolic compounds from the olive paste and pomace were extracted following the modified method of Fantozzi and Montedoro, as reported by Chimi and Atouati [17] with some modifications. Briefly, 200 g of each sample were crushed with a refrigerated cleaver mill for 3 min in order to obtain a homogenous paste. Extrac-

tion, purification and separation were done as follows: 4 g sample was extracted with 80 mL 80% vol/vol ethanol-water mixture containing sodium metabisulfite (400 mg/kg). The mixture was homogenized using a Polytron homogenizer, centrifuged at 1685 × g for 5 min and filtered under vacuum conditions. Then, the remaining ethanol extract was evaporated under vacuum at 31 °C to a volume of 1–2 mL (a syrupy consistence). The purification was carried out with 120 mL acid methanol and 40 mL *n*-hexane, repeated three times. The final methanol extract was evaporated at 31 °C and the phenolic extract was re-diluted in methanol for HPLC analysis.

The phenols were extracted from the olive oil following the procedure of Montedoro et al. [18].

The wastewater was separated from the oil by decanting and filtering under vacuum. The resulting extract was filtered through a 0.45-μm filter. All the extraction procedures were done in triplicate.

2.5 HPLC analysis of phenolic compounds

The phenolic fraction extracted from olive paste, pomace and olive oil was dissolved in 1 mL methanol and analyzed by HPLC (loop 20 μL). The wastewater was directly injected into the chromatograph based on the method reported by Romero et al [19].

The HPLC system consisted of a Waters 600 E pump, a Waters column heater module, a Waters 717 plus auto sampler and a Waters 996 photodiode array detector (PDA) (Waters Inc., Milford, MA, USA). The column was an Inertsil ODS-3 (5 μm, 15 cm × 4.6 mm i.d.; GL Sciences Inc., Tokyo, Japan) equipped with a Spherisorb S5 ODS-2 (5 μm, 1 cm × 4.6 mm i.d.; Technokroma, Barcelona, Spain) pre-column. HPLC analysis was performed following the same procedure as Montedoro et al. [18]. The chromatograms were extracted at 278 nm and 339 nm. Empower Software 2002 (Waters Corporation, Milford, USA) was used to manage the system and to process the information.

2.6 Reference compounds

Tyrosol and *p*-coumaric acid were obtained from Extrasynthèse Co. (Genay, France). Vanillic acid and vanillin were obtained from Fluka Co. (Buchs, Switzerland). Hydroxytyrosol was obtained by acid hydrolysis of oleuropein glucoside [20]. The rest of the phenolic compounds were obtained using a semi-preparative HPLC column Spherisorb ODS-2 (5 μm, 25 cm × 10 mm i.d.; Waters, USA) and a flow rate of 4 mL/min. The mobile phases and the gradient are described elsewhere [7]. Individual phenols were quantified by a four-point regression curve on the basis of the standards obtained from commercial suppliers or from semi-preparative HPLC as described above. Quantification of the phenolic compounds of olive paste, pomace, olive oil and wastewater was carried out at 278 nm and 339 nm.

3 Results and discussion

3.1 The phenolic profile of solid and liquid phases

Fig. 1 shows the chromatographic profile of the phenolic compounds in the olive paste and the corresponding pomace, olive oil and waste water, and Tab. 1 shows the tentatively identified phenolic compounds in the solid and liquid phases and their retention times.

Tab. 1. Summary of the identified phenolic compounds and retention times in the solid and liquid phases obtained during the oil extraction process.

Peak No.	Retention time [min]	Phenolic compound
1	10.6	3,4-DHPEA 4-β-D-glucoside
2	11.1	3,4-DHPEA
3	11.9	3,4-DHPEA specie
4	14.2	*p*-HPEA
5	15.8	Peak unknown
6	17.9	Vanillic acid
7	18.4	Homovanillic acid
8	19.7	Vanillin
9	22.6	3,4-DHPEA-AC
10	22.9	Demethyloleuropein
11	25.0	Verbascoside
12	26.6	3,4-DHPEA-EDA
13	28.5	Luteolin-7-O-glucoside
14	29.1	Rutin
15	29.6	Oleuropein
16	30.1	Apigenin-7-O-glucoside
17	30.9	*p*-HPEA-EDA
18	31.9	Lignans (acetoxypinoresinol + pinoresinol)
19	32.2	Peak unknown
20	32.5	*p*-HPEA-EA
21	36.1	3,4-DHPEA-EA
22	37.2	Luteolin
23	38.5	Apigenin

[#] 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.

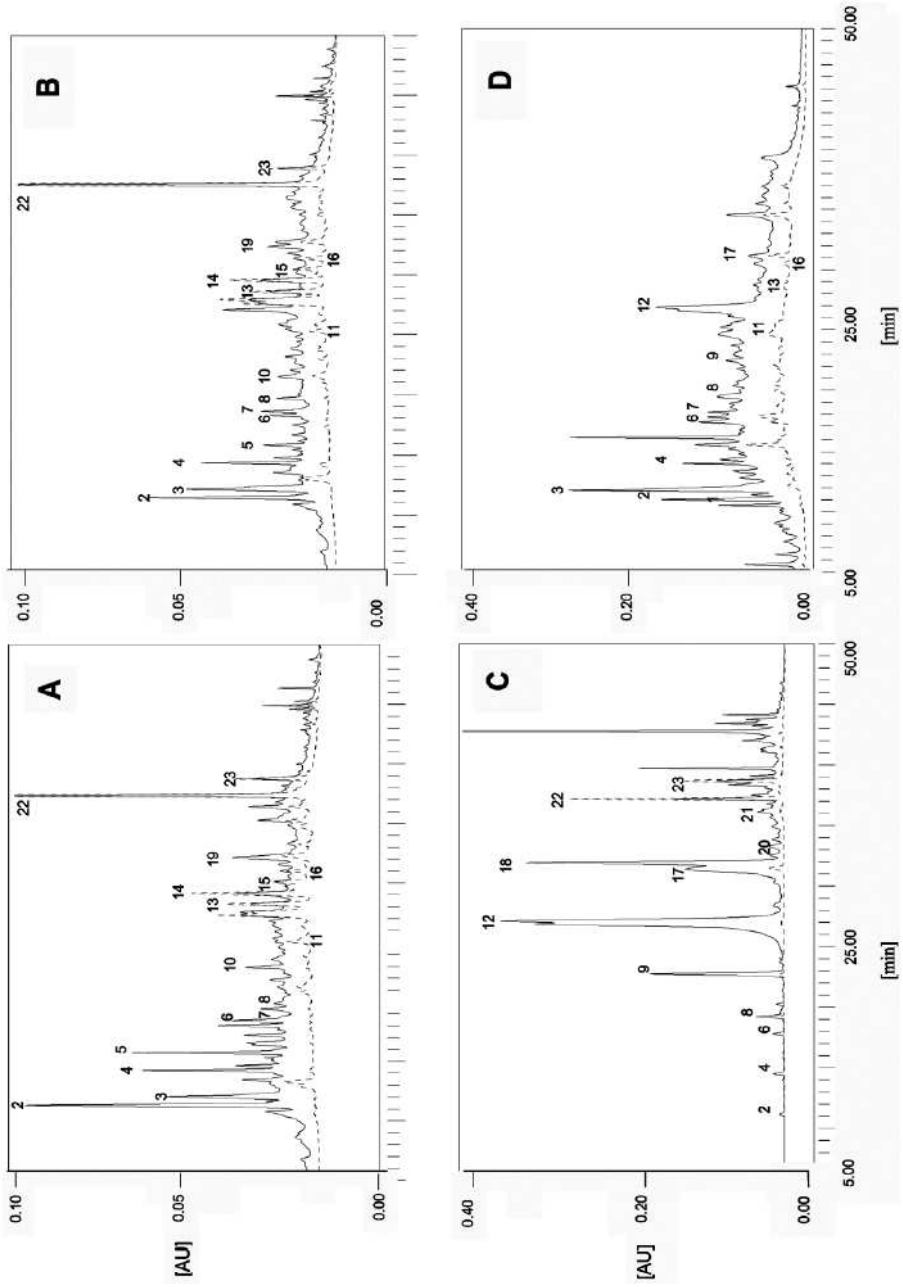

Fig. 1. HPLC chromatograms, at 278 (—) and 339 nm (- - -), of phenolic extracts from olive paste (A), pomace (B), olive oil (C) and wastewater (D). See Tab. 1 to identify the peaks.

The HPLC profile of the olive paste was similar to that of the pomace. The predominant phenolic compounds of these phases were secoiridoid oleuropein and other classes of phenolic compounds including phenyl alcohols, such as hydroxytyrosol (3,4-DHPEA) and tyrosol (*p*-HPEA), phenyl acids, such as vanillic and homovanillic acids, and vanillin. Peaks 2 and 5 could be considered simple phenols as they showed similar UV spectrum characteristics and due to their retention time. Moreover, peak 19 presented similar UV spectrum characteristics to those of oleuropein and was included in the oleoside group. Flavonoids constitute the rest of the phenolic compounds quantified. Secoiridoid aglycons, which originate from oleuropein and demethyloleuropein during mechanical oil extraction, were not detected in the crushed paste.

The phenolic compounds found in the olive oil were in accordance with those reported from the Arbequina cultivar in other studies by our research group [7–8, 21]. The olive oil showed low amounts of phenyl acids and phenyl alcohols, the prevalent phenolic compounds being the secoiridoid derivatives, such as the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), the dialdehydic form of elenolic acid linked to tyrosol (*p*-HPEA-EDA), the aldehydic form of elenolic acid linked to tyrosol (*p*-HPEA-EA) and oleuropein aglycon (3,4-DHPEA-EA), and lignans. The appearance of these compounds in oil raises the issue of the degradative pathways of the phenolic oleosides. It has been assumed that crushing and malaxation of the olive fruits during oil production activates the endogenous β-glucosidases, which produces the aglycones [22].

Most of the phenolic compounds found in the oil were also present in the wastewater, except for the less polar ones which elute at retention times greater than 32 min. The prevalent phenolic compound in the wastewater was the secoiridoid derivative 3,4-DHPEA-EDA, although high concentrations of phenyl alcohols were also found. The 3,4-DHPEA 4-β-D-glucoside and verbascoside were only quantified in the wastewater. This phase also contained glycosidic forms of flavonoids, luteolin-7-glucoside and apigenin-7-glucoside that were not detected in the oil.

3.2 Effect of irrigation treatment on the partitioning of phenolic compounds

The phenolic compound contents of olive paste from non-irrigated and irrigated olive trees and their partitioning into the pomace, oil and wastewater are shown in Tab. 2. The results are expressed as mg of each phenol related to 1 kg of olive paste from non-irrigated and irrigated olive trees, respectively.

The compound 3,4-DHPEA 4-β-D-glucoside was only detected and quantified in the wastewater, independently of the origin of the sample (irrigated, non-irrigated). It is a very polar compound. In fact, it is much more polar than hydroxytyrosol; therefore, it is reasonable that it should not be found in the oil phase. This compound can be found either as a simple phenol or esterified with elenolic acid to form oleuropein and its aglycon, and as a part of the verbascoside molecule [19]. If the amount of this phenolic compound in the olive paste was not detectable, it could be formed during processing.

The most abundant simple phenols, 3,4-DHPEA and *p*-HPEA, were affected by irrigation and showed the same trend. The content of these two phenyl alcohols was significantly higher in samples from non-irrigated trees. However, the most remarkable was their partitioning into the pomace and wastewater. While the highest proportion of them partitioned into the pomace in the samples from non-irrigated trees, in those from irrigated trees most of them was lost in the wastewater. The proportion of phenolic compounds residing in the different phases depends on their relative polarities and the relative amount of the phase. The solubility of 3,4-DHPEA and *p*-HPEA in the aqueous phase is higher than in the oil phase, with partition coefficients (oil/water) of 0.01 and 0.08, respectively [11]. These results may be due to the different quantities of the water in the olive paste. The initial water content of the samples from irrigated trees is significantly higher than of the samples from the non-irrigated trees (61.7 and 47.1%, respectively), and since the amount of water added during the oil extraction process is the same, the volume of the aqueous phase is higher in the samples from the irrigated trees. The amount of water modifies the concentration of the soluble substances in the oil and aqueous phases obtained during malaxation. During this operation, the phenolic compounds dissolve into the oil and the aqueous immiscible phases in contact, according to the corresponding value of the partition coefficients.

The 3,4-DHPEA content was also higher in samples from non-irrigated trees. However, there was an increase in the wastewater in samples from irrigated trees in relation to the initial content in the olive paste. It could be supposed that this amount was enriched by hydrolysis of secoiridoid derivatives and verbascoside. Peak 5 (retention time 15.8 min in the solid phases) was considered a simple phenol according to its spectral characteristics and could only be quantified in the solid phases from non-irrigated trees. In the liquid phases, it was not detected in samples from non-irrigated trees or irrigated trees.

Phenolic acids and vanillin were not greatly affected by irrigation. Vanillin and vanillic acid did not differ in the samples from non-irrigated and irrigated trees, although

Tab. 2. Partitioning of phenolic compounds (expressed as mg per kg of olive paste) during the olive oil extraction process, comparing olives from non-irrigated and irrigated olive trees. See Tab. 1 for abbreviations of the phenolic compounds.

Phenolic compounds	Non-irrigated				Irrigated			
	Olive paste	Pomace	Oil	Wastewater	Olive paste	Pomace	Oil	Wastewater
3,4-DHPEA 4-β-D-glucoside	ND	ND	ND	10.47	ND	ND	ND	6.45
3,4-DHPEA	95.00	65.17	0.09	28.85	42.54	9.52	0.10	25.06
3,4-DHPEA specie	101.00	34.29	ND	37.12	12.17	5.03	ND	36.26
p-HPEA	100.55	43.41	0.18	35.16	37.01	8.11	0.11	22.26
Peak 15.8 min	48.73	27.66	ND	ND	TR	TR	ND	ND
Total simple phenols	345.28	170.53	0.27	111.60	91.72	22.66	0.21	90.03
Vanillic acid	18.42	5.59	0.05	2.69	14.21	5.58	0.07	3.66
Homovanillic acid	28.03	13.69	ND	7.09	9.08	3.95	ND	4.49
Total phenolic acids	46.45	19.28	0.05	9.78	23.29	9.53	0.07	8.15
3,4-DHPEA-EDA	ND	ND	136.24	630.81	ND	ND	4.64	16.92
p-HPEA-EDA	ND	ND	9.83	TR	ND	ND	1.20	ND
p-HPEA-EA	ND	ND	0.61	TR	ND	ND	0.28	ND
3,4-DHPEA-EA	ND	ND	11.53	TR	ND	ND	ND	ND
Total secoiridoid derivatives			158.21	630.81			6.12	16.92
Demethyloleuropein	TR	TR	ND	ND	TR	TR	ND	ND
Oleuropein	31.00	9.45	ND	ND	TR	TR	ND	ND
Peak 32.2 min	58.78	12.36	ND	ND	13.58	ND	ND	ND
Total oleosides	89.78	21.81			13.58			
Luteolin-7-glucoside	45.05	27.09	ND	10.37	16.97	10.03	ND	3.05
Rutin	107.00	56.99	ND	ND	25.67	5.48	ND	ND
Apigenin-7-glucoside	7.55	2.73	ND	2.19	2.82	1.14	ND	1.09
Luteolin	124.12	107.79	0.88	ND	28.55	24.60	0.16	ND
Apigenin	5.32	2.94	0.27	ND	1.21	1.11	0.06	ND
Total flavonoids	289.04	197.54	1.15	12.56	75.22	42.36	0.22	4.14
Vanillin	15.72	24.64	0.06	TR	20.32	17.44	0.04	ND
3,4-DHPEA-AC	ND	ND	19.7	9.9	ND	ND	9.86	TR
Lignans	ND	ND	10.28	ND	ND	ND	5.65	ND
Verbascoside	TR	TR	ND	6.53	TR	TR	ND	5.73

TR, trace amounts; ND, not detected.

the homovanillic acid content was higher in samples from non-irrigated trees. The partitions for these phenolic compounds were similar in samples from non-irrigated and irrigated trees.

Secoiridoid derivatives represented the main phenolic fraction in oil and wastewater where they were detected. They were responsible for the decrease of total phenolic content in samples from irrigated trees. The partition coefficients of 3,4-DHPEA-EDA and 3,4-DHPEA-EA were 0.19 and 1.5, respectively, according to Rodis et al. [11], thus suggesting a considerable solubility in the oil phase, especially of oleuropein aglycone which was only detected in the oil. The secoiridoid derivatives are supposed to be degradation products of the oleosides, formed during the oil extraction process. Oleuropein was not present in a quantifiable amount in samples from irrigated trees; this could be the reason for the drastic diminution of the secoiridoid derivative contents in the oil from the irrigated trees. The characteristics of Peak 19 (retention time 32.2 in the solid phases) were similar to those of the secoiridoid compounds, and its presence in the olive paste in samples from irrigated trees could explain the presence of secoiridoid derivative products in the oil of those samples.

Flavonoids are another important group of phenolic compounds found in the different phases of the oil extraction process. The flavonoid content was noticeably higher in the samples from non-irrigated trees than from irrigated trees. Rutin was detected in the solid phases, while luteolin and apigenin were found in all the phases apart from the wastewater. Although their content was higher in the samples from the non-irrigated trees, partitioning between the different phases was similar for the samples from irrigated and non-irrigated trees.

The compound 3,4-DHPEA-AC was only detected in the liquid phases. It was present in higher amounts in oils from non-irrigated than irrigated trees and there were no quantifiable amounts of it in the wastewater from the samples from irrigated trees. Lignans were only quantified in oil and verbascoside in wastewater, with no remarkable differences between samples from irrigated and non-irrigated trees.

3.3 Effect of adding NMT on the partitioning of phenolic compounds

The contents of phenolic compounds in olive paste with and without the addition of NMT and their partitioning into the pomace, oil and wastewater are shown in Tab. 3. The results are expressed as mg of each phenol per kg of olive paste.

Not all the phenolic compounds quantified in the different phases were affected by the addition of NMT during the malaxation step. Among the simple phenols, there were no significant changes in the partitioning into the different phases for 3,4-DHPEA 4-β- D -glucoside and peak 5 (retention time 15.8 min in the solid phases). However, a significant trend was observed for 3,4-DHPEA, 3,4-DHPEA specie and *p*-HPEA. When comparing the samples with the addition of NMT to those without the addition of NMT, a slight decrease in the contents of these phenolic compounds in the pomace and a more marked increase in their contents in the wastewater were observed. Nevertheless, the oils were not enriched with these antioxidant compounds.

During the extraction process, stable emulsions between oil droplets, water droplets and vegetable colloids (made up of hemicellulose, protein, pectin, *etc.*) are formed. The malaxation operation, inducing coalescence phenomena,

Tab. 3. Partitioning of phenolic compounds (expressed as mg per kg of olive paste) comparing the oil extraction process without (NMT–) and with (NMT+) addition of natural micro-talc. See Tab. 1 for abbreviations of the phenolic compounds.

Phenolic compounds	NMT–				NMT+			
	Olive paste	Pomace	Oil	Wastewater	Olive paste	Pomace	Oil	Wastewater
3,4-DHPEA 4-β- D -glucoside	ND	ND	ND	9.50	ND	ND	ND	15.02
3,4-DHPEA	93.77	69.57	0.08	23.54	95.00	65.17	0.09	41.40
3,4-DHPEA specie	102.36	50.43	ND	34.62	101.00	34.29	ND	53.26
p-HPEA	82.84	110.45	0.14	30.23	100.55	43.41	0.18	50.45
Peak 15.8 min	48.26	34.46	ND	ND	48.73	27.66	ND	ND
Total simple phenols	327.23	264.91	0.22	97.89	345.28	170.53	0.27	160.13
Vanillic acid	12.58	5.04	0.05	TR	18.42	5.59	0.05	3.87
Homovanillic acid	31.95	13.66	ND	12.38	28.03	13.69	ND	10.17
Total phenolic acids	44.53	18.7	0.05	12.38	46.45	19.28	0.05	14.04
3,4-DHPEA-EDA	ND	ND	107.46	452.47	ND	ND	136.24	905.07
p-HPEA-EDA	ND	ND	7.29	TR	ND	ND	9.83	TR
p-HPEA-EA	ND	ND	0.41	ND	ND	ND	0.61	ND
3,4-DHPEA-EA	ND	ND	6.22	ND	ND	ND	11.53	ND
Total secoiridoid derivatives			121.38	452.47			152.21	905.07
Demethyloleuropein	TR	TR	ND	ND	TR	TR	ND	ND
Oleuropein	35.19	ND	ND	ND	31.00	9.45	ND	ND
Peak 32.2 min	49.81	ND	ND	ND	58.78	12.36	ND	ND
Total oleosides	85.00				89.78	21.81		
Luteolin-7-glucoside	68.36	28.13	ND	8.24	45.05	27.09	ND	14.88
Rutin	98.28	58.01	ND	ND	107.00	56.99	ND	ND
Apigenin-7-glucoside	6.86	2.86	ND	1.32	7.55	2.73	ND	3.15
Luteolin	106.43	89.18	0.78	ND	124.12	107.79	0.88	ND
Apigenin	4.80	2.33	0.20	ND	5.32	2.94	0.27	ND
Total flavonoids	280.23	180.51	0.98	9.56	289.04	197.54	1.15	18.03
Vanillin	14.02	25.89	0.06	TR	15.72	24.64	0.06	TR
3,4-DHPEA-AC	ND	ND	11.51	TR	ND	ND	13.53	TR
Lignans	ND	ND	8.23	ND	ND	ND	10.28	ND
Verbascoside	TR	ND	ND	5.23	TR	ND	ND	9.37

TR, trace amounts; ND, not detected.

causes the minute bound oil droplets to merge into large drops, thus separating them from both colloids and water droplets. In addition, this step disrupts a proportion of the oil cells that had remained uncrushed during the first step (crushing), allowing the recovery of another oil fraction [13]. The formation of emulsions, which are too stable to be removed by mechanical means, may be involved in the loss of phenolic compounds during processing. The addition of NMT may reduce the stability of the emulsions by absorbing part of the water, reducing the complexation of the phenolic compounds trapped in the emulsion and by improving their release into the oil and wastewater during the extraction process. The addition of NMT increased the release of simple phenols (which show a higher affinity for the aqueous phase) into the wastewater.

In relation to vanillin and phenolic acids, only vanillic acid was affected by the addition of NMT, with its concentration in the wastewater increasing.

Among the secoiridoid derivatives, only 3,4-DHPEA-EDA, the main phenolic compound in the liquid phases, was affected by the addition of NMT during the oil extraction process; its concentration showed an important increase in the wastewater. There was a significant amount of oleosides in the pomace when NMT was added; in contrast, they were not detectable without addition of NMT. The addition of NMT might have improved the extraction of those compounds from the olive paste, implying an increase in their concentration in the pomace and thus the formation of more secoiridoid derivatives, such as hydrosoluble 3,4-DHPEA-EDA. This could result in their sizable loss in the wastewater during processing.

None of the flavonoids quantified in the different phases, 3,4-DHPEA-AC, lignans and verbascoside, were affected by the addition of NMT during the oil extraction process.

According to the results observed in this study, it is possible to conclude that the irrigation applied to olive trees leads to a considerable decrease in the olive paste's phenol content. The water status of the tree affects 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. The most remarkable point of the phenol partitioning concerned the simple phenols. While the biggest proportion of them partitioned into the pomace in samples from non-irrigated trees, in samples from irrigated trees, part of the phenol compounds was lost in the wastewater.

After comparison of the results obtained from the experiments with and without NMT addition, it was concluded that the use of this co-adjuvant did not significantly alter either the phenolic profile of the oil phase obtained or the content of the individual phenolic compounds.

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