Effect of Refining on the Phenolic Composition of Crude Olive Oils

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ABSTRACT: By definition, virgin olive oil is consumed unrefined, although a great proportion of the olive oil produced has to be refined to render it edible. Phenolic compounds are among the substances eliminated during the refining process; in the present work these were characterized by HPLC, and their evolution during the different refining steps was studied. The complete refining process removed most polyphenols from oils, but the behavior of individual compounds at each step also was observed. o-Diphenols (hydroxytyrosol, catechol, and hydroxytyrosol acetate) and flavonoids (luteolin and apigenin) were eliminated first during the alkaline treatment. Tyrosol and 4-ethylphenol remained in the oil until the deodorization step. A large amount of phenolic compounds was discovered in the refining by-products such as soapstocks and deodorization distillates. In the latter streams, the concentrations of tyrosol and 4-ethylphenol reached up to 149 and 3720 mg/kg by-product, respectively. This high level of 4-ethylphenol and its well-known strong off-odor can interfere during further processing of the deodorization distillates, and this must be taken into account when deciding what is to become of them. Similarly, the results of this work open the possibility of recovering phenolic compounds from the "second centrifugation olive oils" by adding a new washing step prior to the refining process. By including this new step, the most polar polyphenols, hydroxytyrosol and tyrosol, will diffuse from oil to water and a concentration of up to 1400 mg/L of hydroxytyrosol may be achieved.

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KEY WORDS: 4-Ethylphenol, hydroxytyrosol, olive oil, phenolic compounds, refining, tyrosol.

Virgin olive oil, which is obtained using only mechanical systems, is a foodstuff that is consumed in its crude (unrefined) form. However, a great proportion of the olive oil that is produced must be refined to render it edible. At present, three types of olive oils are intended for refining: lampante olive oil, olive-pomace oil, and second centrifugation olive oil. Lampante olive oil is obtained from fruits by mechanical means, but it has undesirable organoleptic or chemical characteristics that make it unfit for consumption. Likewise, the olive paste obtained during the dual-phase centrifugation system used for olive oil extraction is stored for months and subjected to chemical extraction with hexane to produce the traditional olive-pomace oil (1), or it can also undergo a new second centrifugation to yield a second centrifugation olive oil (2).

Refining treatments are needed to remove or reduce the content of minor substances that may affect oil quality, such as phospholipids, FFA, pigments, peroxides, traces of metals, herbicides, and volatile components (3,4). Phenolic compounds also are removed during refining (5,6). In particular, the total polyphenol content of olive oil determined colorimetrically is reduced almost to zero with refining (7,8). Nergiz (9) reported that a residual amount of total polyphenols and *o*-diphenols can be found in refined olive oils, although Cortesi *et al.* (10) did not detect them.

Recently, we analyzed the polyphenol content of crude olive oils that were intended for refining by HPLC (11) and found a considerable amount of these substances, particularly in second centrifugation olive oils. Polyphenols can be used for food, cosmetic, or pharmaceutical purposes (12), and information on the concentration of these compounds during oil refining is required. The recovery of valuable compounds such as these from by-products generated during oil refining, including soapstocks (13) and deodorization distillates (14,15), is an emerging industry.

The objectives of the present study were (i) to evaluate the influence of the different refining steps on the polyphenol content of crude oils and (ii) to evaluate their content in the by-products generated in this process.

MATERIALS AND METHODS

Industrial samples. Crude lampante olive oil, olive-pomace oil, and second centrifugation oil were industrially refined (Oleícola el Tejar S.L., Córdoba, Spain) following the common process described in Scheme 1. Samples were kept frozen in dark glass bottles at −30°C until chemical analyses were performed.

Chemical laboratory refining. Oils were degummed with acidified water (0.2% phosphoric acid) for 30 min at 25°C. Then, a sufficient amount of NaOH (18° Bé) was added *in situ* to neutralize the FFA and the added mineral acid, plus 10% more to ensure the displacement of the reaction toward the formation of soaps at 80°C for 20 min. The pastes were then separated by centrifugation at 2000 × *g* for 10 min. Subsequently, 5% (w/w) of diluted NaOH (4° Bé) was added to the unwashed, degummed, and neutralized oils, and these were kept at 9°C for 13 h. Waxes and saturated TG were removed from the samples

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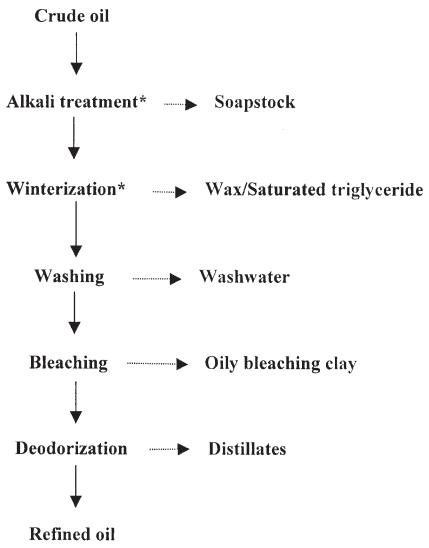

*Not applied for "lampante olive oil"

SCHEME 1

by centrifuging at 2000 × *g* for 10 min. To eliminate traces of soaps dissolved in the oil and the remains of the mineral reagents added, the oils obtained in the previous stages were washed in an agitation tank reactor, with four successive additions of 10% water by weight. The four washing waters were removed by centrifugation, the first at room temperature and the other three at 90°C.

Physical refining trials at pilot plant scale. Replicated trials were carried out with 200-kg lots of lampante olive oil, which were previously washed with 10% water at 80°C and bleached in the pilot plant under the following conditions: clay, 1% (Fulmont; Süd Chemie AG, Moosburg, Germany); temperature, 90°C; and time, 30 min. For each experiment, the deodorizer was filled with 200 kg of homogeneous bleached oil, which was then preheated from 30°C to the required temperature. The test temperatures were 180 and 240°C. Time was recorded when the oil reached the test temperature. Steam was introduced when the oil reached 100°C, to ensure satisfactory heat transfer and protection of the product, and then refining began. The rate of stripping steam was adjusted to 2%/h. After 3 h, the batch was cooled. The steam flow was terminated when the oil temperature reached 100°C. A sample of the final cooled oil was taken.

Comparison of oil extraction methods. Two samples of olive paste stored for 8 mon were extracted in the industrial oil factory by two different methods, either by using a second centrifugation system or by using hexane as solvent. Samples of oils were analyzed for the presence of phenolic compounds.

Trials of washing crude oils before refining. The preceding two samples of oils obtained from the second centrifugation process were mixed with tap water in a ratio of 10:1 and 10:5 (g oil/mL water), agitated vigorously at room temperature for 1 min in a vortex, and centrifuged for 5 min at 12,800 × *g*. The aqueous phases were passed through a 0.45 μm nylon filter, and 20 μL was directly injected into the chromatograph for polyphenols analysis.

Extraction of phenolic compounds from oils. Phenolic extracts of olive oils were obtained following the procedure described elsewhere (16). Briefly, 0.6 mL of olive oil was extracted using 3 × 0.6 mL of *N,N*-dimethylformamide (DMF); the extracts were combined and then washed with hexane, and N_2 was bubbled into the DMF extract to eliminate residual hexane. Finally, the extract was filtered through 0.45 μm pore size filter and injected into the liquid chromatograph.

Extraction of phenolic compounds from deodorization distillates. Samples (0.6 g) were diluted with 3.2 mL of hexane and phenolic compounds extracted as described herein.

HPLC analysis of phenolic compounds. The chromatographic system consisted of a Waters 717 Plus Autosampler, a Waters 600E pump, and a Waters column heater module (Waters Inc., Milford, MA). A Spherisorb ODS-2 (5 μm, 25 cm × 4.6 mm i.d., Waters Inc.) column was used. Separation was achieved using an elution gradient with an initial composition of 90% water (pH adjusted to 3.0 with phosphoric acid) and 10% methanol. The concentration of the latter solvent was increased to 30% over 10 min and maintained for 20 min. Subsequently, the methanol percentage was raised to 40% over 10 min, maintained for 5 min, and then increased to 50%. Finally, the methanol percentage was increased to 60, 70, and 100% in 5-min periods. Initial conditions were reached in 15 min. A flow of 1 mL/min and a temperature of 35°C were used in all of the experiments. A Waters 996 diode array detector and a JASCO FP-920 fluorescence detector (JASCO, Tokyo, Japan) were connected in series. Vanillin, the dialdehydic form of decarboxymethyl oleuropein aglycon, and vanillic and *p*-coumaric acids were monitored by UV at 280 nm, luteolin and apigenin at 340 nm, and the rest of the phenolic compounds by fluorescence with an excitation wavelength at 280 nm and an emission wavelength at 320 nm. Both detectors were operated with Millenium 2015 software (Waters Inc.). Quantification of phenolic compounds was made by using reference compounds obtained from commercial suppliers or from preparative HPLC as described elsewhere (17).

HPLC–MS analysis. All phenolic extracts were analyzed by LC–MS using a ZMD4 mass spectrometer (Waters Inc.) equipped with an electrospray ionization probe, and working in the negative ion mode. Cone voltage fragmentation was 20 V, capillary voltage, 3 kV, desolvation temperature 250°C, source temperature 80°C, and extractor voltage, 12 V. A constant flow of 1 mL/min was used for each analysis with a split ratio of approximately 5:1 (UV detector/MS detector).

RESULTS AND DISCUSSION

Simple phenols were the main polyphenols characterized by HPLC-MS in the phenolic extracts of crude oils (Tables 1–3), except for lampante olive oils that also contained low concentrations of oleuropein and ligstroside aglycons (11). These aglycons

TABLE 1
Effect of the Refining Steps on the Phenolic Concentration (mg/kg oil) of a Lampante Olive Oil

Polyphenol	Crude oil	Washed oil	Bleached oil	Deodorized oil	Deodorization distilled[a]
Hydroxytyrosol	2.1 (0.1)[b]	0.1 (0.1)	ND	ND	ND
Catechol	4.1 (0.3)	0.6 (0.1)	0.6 (0.1)	ND	10.8 (3.1)
Tyrosol	3.0 (0.1)	2.6 (0.1)	2.4 (0.1)	ND	149.1 (30.2)
Hy-AC	2.3 (0.1)	1.8 (0.1)	0.2 (0.1)	ND	ND
4-Ethylphenol	1.5 (0.1)	1.4 (0.1)	1.9 (0.2)	ND	50.2 (7.9)
1-Acetoxypinoresinol	8.5 (0.1)	5.4 (0.2)	1.2 (0.3)	1.5 (0.2)	ND
Pinoresinol	13.7 (0.1)	12.0 (0.4)	2.4 (0.1)	2.6 (0.1)	6.6 (3.0)
Hy-EA	14.7 (0.5)	ND	ND	ND	ND
Ty-EA	7.3 (1.8)	ND	ND	ND	ND
Luteolin	3.1 (0.3)	ND	ND	ND	3.5 (0.3)
Apigenin	1.3 (0.1)	ND	ND	ND	1.5 (0.1)

[a]This residue represented 0.16% of the bleached oil.
[b]SD of two analyses. ND, not detected; Hy-AC, acetylated hydroxytyrosol; Hy-EA, oleuropein aglycon; Ty-EA, ligstroside aglycon.

TABLE 2
Effect of the Refining Steps on the Phenolic Concentration (mg/kg oil) of an Olive-Pomace Oil

Polyphenol	Crude oil	Washed oil[a]	Bleached oil	Deodorized oil	Deodorization distilled[b]
Hydroxytyrosol	93.6 (5.5)[c]	0.2 (0.1)	ND	ND	ND
Catechol	17.1 (2.3)	0.2 (0.1)	ND	ND	ND
Tyrosol	29.3 (1.0)	3.1 (0.1)	0.2 (0.1)	ND	52.4 (3.7)
Hy-AC	70.7 (15.4)	27.9 (0.5)	ND	ND	ND
4-Ethylphenol	15.1 (0.1)	10.7 (0.1)	7.9 (0.3)	ND	1009.8 (64.2)
1-Acetoxypinoresinol	80.8 (0.5)	7.7 (0.8)	ND	ND	ND
Pinoresinol	88.6 (0.6)	23.1 (0.4)	ND	ND	ND
Vanillin	4.7 (0.2)	ND	ND	ND	ND

[a]This oil was alkali-treated, winterized at <4°C, and washed with tap water.
[b]This constituted 1.05% of the bleached oil.
[c]SD of two analyses. For abbreviations see Table 1.

are the main polyphenols in virgin olive oils (18,19) together with the lignans 1-acetoxypinoresinol and pinoresinol (17).

Trials carried out at the industrial level confirmed the complete disappearance of polyphenols from oils during the refining process because they were not detected in deodorized oils (Tables 1–3). This means that polyphenols present in commercial olive oils (20) come mostly from the virgin olive oil blended with refined oil. Although there are previous reports on the loss of olive polyphenols during refining of lampante olive oil (7,8), this is the first time that they have been studied by the HPLC technique in all types of crude olive oils intended for refining. That residual amounts of some polyphenol derivatives such as oryzanol are found in deodorized rice bran oil (5) raised the possibility of a similar situation for olive oils, but these were not observed.

Interestingly, most simple phenols except tyrosol and 4-ethylphenol were lost before the deodorization step. The *o*-diphenols hydroxytyrosol, catechol, and hydroxytyrosol acetate were

TABLE 3
Effect of the Refining Steps on the Phenolic Concentration (mg/kg oil) of a Second Centrifugation Olive Oil

			Deodorization		
Polyphenol	Crude oil	Washed oil[a]	Bleached oil	Deodorized oil	distilled[b]
Hydroxytyrosol	346.7 (24.1)[c]	ND	ND	ND	ND
Catechol	97.5 (1.9)	ND	ND	ND	ND
Tyrosol	50.5 (0.4)	0.8 (0.1)	1.6 (0.1)	ND	62.5 (0.1)
Hy-AC	145.0 (0.7)	ND	ND	ND	ND
4-Ethylphenol	262.2 (1.9)	211.9 (12.2)	174.2 (1.3)	0.2 (0.1)	3720.5 (321.7)
1-Acetoxypinoresinol	54.2 (8.3)	11.6 (6.7)	ND	ND	ND
Pinoresinol	18.9 (2.6)	5.9 (0.6)	ND	ND	ND
Luteolin	9.6 (2.4)	ND	ND	ND	ND
Apigenin	5.4 (0.5)	ND	ND	ND	ND

[a]This oil was alkali-treated, winterized at a temperature <4°C, and washed with tap water.
[b]This constituted 2.0% of the bleached oil.
[c]SD of two analyses. For abbreviations see Table 1.

TABLE 4
Effect of the Neutralization, Winterization, and Washing Steps on the Phenolic Composition of a Second Centrifugation Olive Oil Obtained from an Olive Paste Stored for 3 mon

Polyphenol	Crude olive (mg/kg)	Soapstock (mg/kg)	Wax[a] (mg/kg)	Washwater 1 (mg/L)	Washwater 2 (mg/L)	Washwater 3 (mg/L)	Washwater 4 (mg/L)	Washed oil (mg/kg)
Hydroxytyrosol	23.4 (0.7)[b]	ND	ND	ND	ND	ND	ND	ND
Catechol	8.0 (0.4)	16.0 (8.1)	ND	ND	ND	ND	ND	ND
Tyrosol	20.0 (0.2)	146.1 (57.1)	3.9 (0.4)	9.8 (0.2)	0.1 (0.1)	ND	ND	ND
Hy-AC	19.7 (1.0)	2.3 (0.3)	ND	ND	ND	ND	ND	ND
4-Ethylphenol	15.6 (0.5)	13.6 (5.8)	9.9 (0.2)	28.3 (3.0)	0.7 (0.1)	0.2 (0.1)	ND	2.9 (0.2)
1-Acetoxypinoresinol	35.4 (0.2)	ND	ND	ND	ND	ND	ND	ND
Pinoresinol	66.9 (0.1)	ND	ND	5.1 (0.2)	ND	ND	ND	ND
Luteolin	3.4 (0.1)	ND	ND	ND	ND	ND	ND	ND
Apigenin	1.0 (0.1)	ND	ND	ND	ND	ND	ND	ND

[a]Waxes and saturated TG.
[b]SD of two analyses. For abbreviations see Table 1.

TABLE 5
Effect of the Neutralization, Winterization, and Washing Steps on the Phenolic Composition of a Second Centrifugation Olive Oil Obtained from an Olive Paste Stored for 8 mon

Polyphenol	Crude olive (mg/kg)	Soapstock (mg/kg)	Wax[a] (mg /kg)	Washwater 1 (mg/L)	Washwater 2 (mg/L)	Washwater 3 (mg/L)	Washwater 4 (mg/L)	Washed oil (mg/kg)
Hydroxytyrosol	135.2 (8.2)[b]	1.5 (0.3)	ND	ND	ND	ND	ND	ND
Catechol	128.2 (8.9)	56.8 (2.6)	ND	ND	ND	ND	ND	ND
Tyrosol	99.2 (6.4)	421.8 (29.2)	94.2 (10.7)	36.8 (4.2)	2.3 (0.2)	1.6 (0.2)	1.1 (0.1)	ND
Hy-AC	80.0 (1.3)	ND	55.9 (1.3)	23.9 (2.5)	1.0 (0.1)	0.5 (0.1)	0.4 (0.1)	ND
4-Ethylphenol	316.3 (11.0)	333.4 (30.4)	231.5 (12.2)	73.7 (4.8)	2.5 (0.2)	1.5 (0.2)	1.4 (0.1)	80.1 (4.6)
1-Acetoxypinoresinol	60.4 (9.1)	ND	ND	ND	ND	ND	ND	ND
Vanillic acid	4.1 (0.2)	6.4 (0.5)	ND	ND	ND	ND	ND	ND
Luteolin	3.9 (0.1)	ND	ND	ND	ND	ND	ND	ND
Apigenin	2.0 (0.1)	ND	ND	ND	ND	ND	ND	ND

[a]Waxes and saturated TG.
[b]SD of two analyses. For abbreviations see Table 1.

preferentially eliminated from oil during the alkaline treatment, winterization, and washing steps. These compounds, particularly hydroxytyrosol and catechol, are very soluble in water and are easy to oxidize under alkaline conditions; the latter could explain their loss during these steps. Surprisingly, the low-hydrophilicity lignans were also removed in a very high proportion during the first refining steps. The flavonoids luteolin and apigenin were completely lost before the oils were bleached.

Because of the important losses in polyphenols occurring during these steps, we performed a step-by-step study of the polyphenols in second centrifugation olive oils during refining; the results are presented in Tables 4 and 5. First, *o*-diphenols (hydroxytyrosol, catechol, and hydroxytyrosol acetate) were mostly eliminated from oil during the alkaline treatment, which was expected. They were probably oxidized and polymerized under these alkaline conditions, as this process is well known, and therefore they were not concentrated in the soapstock streams. On the other hand, tyrosol was accumulated in the soapstocks and a significant amount of 4-ethylphenol was also detected.

The subsequent winterization and washing steps removed polyphenols from oils but to a lesser extent than the alkaline treatment (Tables 4 and 5). The most recalcitrant polyphenol was 4-ethylphenol, which remained in the oil even after four washing periods. These extreme refining conditions are usually applied in industry for second centrifugation olive oils because of their high acidity and their high content of undesirable compounds.

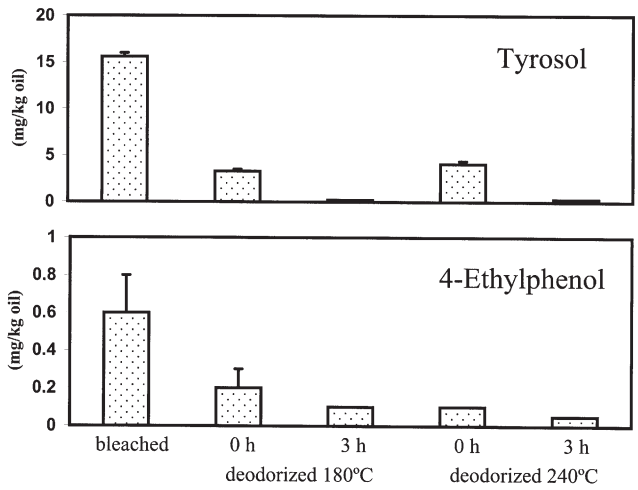

FIG. 1. Effect of deodorization temperature and time on the phenolic content of oil refined physically in the pilot plant.

TABLE 6
Influence of the Extraction Method on the Polyphenol Content (mg/kg oil) of Oil[a]

	Sample A		Sample B	
Polyphenol	Hexane	Centrifugation	Hexane	Centrifugation
Hydroxytyrosol	0.7 (0.1)[b]	126.6 (5.5)	99.6 (11.5)	366.4 (11.2)
Catechol	1.8 (0.1)	91.6 (11.2)	28.5 (0.7)	53.5 (2.7)
Tyrosol	37.3 (3.6)	69.8 (3.0)	173.0 (20.5)	94.1 (10.4)
4-Ethylphenol	67.3 (6.6)	672.1 (6.1)	93.5 (9.8)	149.8 (4.3)
Vanillic acid	5.4 (0.4)	5.3 (1.5)	24.5 (9.8)	9.9 (4.3)
Luteolin	ND	8.0 (2.4)	ND	25.1 (4.3)
Apigenin	ND	2.2 (0.5)	ND	9.4 (2.0)

[a]The residual oil in two olive pastes stored for 8 mon was extracted with hexane or by centrifugation.
[b]SD of two analyses. For abbreviation see Table 1.

TABLE 7
Influence of the Oil/Water Ratio on the Phenolic Composition (mg/L) of the Wash Water[a]

	Sample A		Sample B	
Polyphenol	10:1[b]	10:5[b]	10:1[b]	10:5[b]
Hydroxytyrosol	322.7 (84.8)[c]	68.4 (13.1)	1346.1 (48.4)	295.7 (13.4)
Catechol	86.2 (11.6)	28.0 (1.0)	18.2 (1.0)	8.4 (0.9)
Tyrosol	213.9 (21.7)	75.4 (0.3)	229.1 (2.8)	84.1 (2.8)
4-Ethylphenol	10.2 (1.2)	9.6 (0.2)	1.6 (0.1)	1.6 (0.2)

[a]Two second centrifugation oils obtained from olive pastes stored for 8 mon were treated with tap water at room temperature.
[b]Ratio oil/water.
[c]SD of two analyses.

Deodorization, which is the final step in refining edible olive oils, is typically achieved at a temperature >180°C. Its purpose is to reduce the content of undesirable volatile compounds (odorous components, peroxides, FFA, pesticides) of the oil to comply with quality requirements of the end product. During this step, most polyphenols were removed from the oil although very low amounts of 4-ethylphenol and lignans were found in some deodorized oils (Tables 1–3).

As deodorization was the key step in the removal of polyphenols, the influence of deodorization time and temperature on the elimination of tyrosol and 4-ethylphenol was studied. Results are illustrated in Figure 1. For the two temperatures (180 and 240°C), as soon as the deodorization process was started, that is, when the set temperature was reached in the oil, the content of tyrosol and 4-ethylphenol fell below half of the initial concentration. The reduction continued at 3 h of deodorization.

As a consequence of this process, a very high concentration of these two phenolic compounds (tyrosol and 4-ethylphenol) was reached in the deodorization distillates (Tables 1– 3). However, this refining stream represented a very small amount of the bleached oil. Thus, the deodorization distillate of the lampante olive oil was only 0.16% of the bleached oil (Table 1), and it contained ~150 mg/kg of tyrosol, which is much higher than the initial content in the crude oil. Similarly, the deodorization distillates of the olive-pomace oil (Table 2) and second centrifugation oil (Table 3) had 4-ethylphenol in concentrations as high as 1009 and 3720 mg/kg, respectively. Therefore, this by-product could be a good source of polyphenols in the future, in particular of tyrosol and 4-ethylphenol, which do not possess antioxidant properties but could be used for other purposes. Also, deodorization distillates of vegetable oils contain very important biological substances such as tocopherols, sterols, terpenoids, squalene, and FFA (14,15), and their recovery is an emerging field of research and industrial investment. To our knowledge, this is the first time that the presence of phenolic compounds in deodorization distillates has been reported and, in particular, in distillates from olive oils.

Two important findings were made in this work: (i) Second centrifugation oils had a very high concentration of phenolic compounds, especially *o*-diphenols, and (ii) these compounds were lost during the chemical refining of oils. Taking into account that oil-refining industries in Spain store olive paste for months before extracting the residual oil and that some of them use the second centrifugation system for this purpose whereas other factories dry the paste and extract the oil with hexane, we studied the effect of the two different extractive methods on the polyphenol content of oil. The results in Table 6 indicate that the second centrifugation system gave rise to higher concentrations of polyphenols than the hexane method. In particular, the concentration of *o*-diphenols was much lower when oil was extracted with hexane, which was probably due to the loss of them during the drying process carried out prior to the extraction step.

This work also found that *o*-diphenols were lost during the chemical refining of oils, mainly during the alkaline treatment. We developed a new step to avoid this. In the laboratory, oils were washed with tap water to simulate a first step in a future refining process. Two different oil/water ratios were tested and results are presented in Table 7. The most polar polyphenols,

hydroxytyrosol, catechol and tyrosol, were concentrated in the water phase. A minor amount of the malodorous 4-ethylphenol was also detected in the water phases. In doing a mass balance between the polyphenols content in the oils before washing (Table 6) and in the wash waters (Table 7), half of the hydroxytyrosol, catechol, and tyrosol initially present in the oil passed into the aqueous phase irrespective of the oil/water ratio used. For analytical purposes, it has been proposed that polyphenols can be extracted from olive oil with water. (21). However, the diffusion of these substances from oil to water depends on compound polarity (22,23), as has been demonstrated in this work.

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