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Phenolic Compounds in Olive Oils Intended for Refining: Formation of 4-Ethylphenol during Olive Paste Storage

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The phenolic composition of "lampante olive oil", "crude olive pomace oil", and "second centrifugation olive oil" was characterized by high-performance liquid chromatography with UV, fluorescence, and mass spectrometry detection. The phenolic profile of these olive oils intended for refining was rather similar to that previously reported for virgin olive oil. However, a new compound was found in these oils, which is mainly responsible of their foul odor. It was identified as 4-ethylphenol by comparison of its UV and mass spectra with those of a commercial standard. Although 4-ethylphenol was discovered in all oils intended for refining, its presence was particularly significant in "second centrifugation olive oils", its concentration increasing with time of olive paste storage. Similar trends were observed for hydroxytyrosol, hydroxytyrosol acetate, tyrosol, and catechol, the concentration of these substances reaching values of up to 600 mg/kg of oil, which makes their recovery for food, cosmetic, or pharmaceutical purposes attractive.

KEYWORDS: Olive oil; phenolic compounds; 4-ethylphenol; refining

INTRODUCTION

Virgin olive oil (VOO) is obtained by mechanical means without any chemical treatment. However, a great proportion of the olive oil consumed worldwide consists of a mixture of refined olive oil and VOO (**Figure 1**).

The technology for VOO extraction in Spain is based on the dual-phase centrifugation system: the kneaded olive paste is separated into oil and a new paste called "alpeorujo", which is stored in the open air and can support a second centrifugation; the oil obtained is labeled "second centrifugation olive oil" (SCOO) (*1*). The oils obtained from this method can also be classified as "olive pomace oil" to comply with the new EC Regulations (*2*, *3*). Alternatively, alpeorujo can be dried and subjected to chemical extraction with hexane to produce the traditional "crude olive pomace oil" (COPO) (*2*). Both SCOO and COPO have to be refined before consumption (*4*), although they represent only a small amount of the olive oil intended for refining. A considerable proportion of the virgin olive oil produced in factories is catalogued as "lampante olive oil" (LOO), which possesses acidity >2.0%, waxes <300 ppm, or sensory defects >2.5, among other limits (*3*). LOO can be mixed with virgin olive oil, but the limiting factor is the flavor of LOO.

On the other hand, there is increasing interest in the phenolic compounds of olive oil because of their sensory (*5*), antioxidant (*6*), and nutritional properties (*7*). Thus, many of them present in virgin olive oil have been identified, and the most significant are derivatives of the secoiridoid aglycons of oleuropein and ligstroside (*8*), hydroxytyrosol acetate (*9*), and the lignans 1-acetoxypinoresinol and pinoresinol (*10*). On the contrary, to our knowledge there are no reports on polyphenols in olive oils intended for refining. Zunin and co-workers (*11*) studied the decrease in polyphenols in olive oil when fruits were infested by the fly *Dacus oleae*, but no research has been carried out on the individual characterization of phenolic compounds in LOO, SCOO, and COPO.

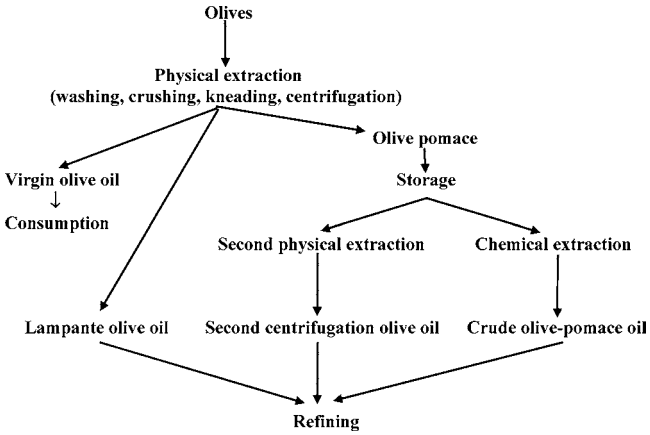

Figure 1. Flux diagram of the processing procedures used to obtain the different types of olive oil.

Although it has been reported that the concentration of polyphenols in oils decreases, to a large extent, during refining (*12*), the study of these substances in crude oils could disclose them as a new valuable source of antioxidants. Therefore, the purpose of this work was (i) to characterize the phenolic

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composition of the olive oils intended for refining and (ii) to evaluate the concentration of polyphenols in crude oils and the influence of time of paste storage.

MATERIALS AND METHODS

Samples. Nine lampante olive oils, two crude olive pomace oils, and nine second centrifugation olive oils were supplied by an olive oil factory (Oleícola el Tejar, Córdoba, Spain). The COPO were obtained from olive pastes stored for 2 months and the SCOO from olive pastes stored for 3 and 8 months.

Extraction of Phenolic Compounds. Phenolic extracts of olive oils were obtained following the procedure described elsewhere (*13*). Briefly, 0.6 mL of olive oil was extracted using 3 × 0.6 mL of *N*,*N*-dimethylformamide (DMF); the extract was then washed with hexane, and N_2 was bubbled into the DMF extract to eliminate the residual hexane. Finally, the extract was filtered through 0.45 μm filters and injected into the chromatograph.

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 the reference compounds obtained from commercial suppliers or preparative HPLC as described elsewhere (*13*).

HPLC-MS Analysis. All phenolic extracts were analyzed by LC-MS using a quadrupole mass analyzer (ZMD4, Waters Inc.) equipped with an ESI probe and working in the negative-ion mode. Cone voltage fragmentation was 20 V, capillary voltage, 3 kV, desolvation temperature, 120 °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).

HPLC Isolation of 4-Ethylphenol and Analysis by GC-MS. 4-Ethylphenol was collected from 20 HPLC analytical injections of the phenolic extracts of a SCOO. Conditions were similar to those reported above, except the aqueous mobile phase did not have phosphoric acid. The compound was extracted from the pooled mobile phase with chloroform (20 mL × 3 times) and injected directly into the GC. GC-MS analysis of the chloroform extract was conducted with a Hewlett-Packard 6890 GC Plus coupled with an Agilent 5973 MSD (mass selective detector, quadrupole type). A fused-silica HP5-MS capillary column (30 × 0.25 mm i.d.; coating thickness = 0.25 mm) was used. Working conditions were as follows: carrier gas, helium (1 mL/min at constant flow); injectorr, 250 °C; oven temperature, from 70 (1 min) to 240 ° C at 5 °C/min and then to 325 °C at 10 °C/min; transfer line to MSD, 280 °C; ionization EI, 70 eV.

A commercial standard of 4-ethylphenol was purchased from Sigma-Aldrich Inc.

RESULTS AND DISCUSSION

Figures 2 and **3** show representative chromatograms of the olive oils studied under UV and fluorescence detections. Most compounds have previously been described in virgin olive oil (*8*, *9*), but their presence was also confirmed in these oils by

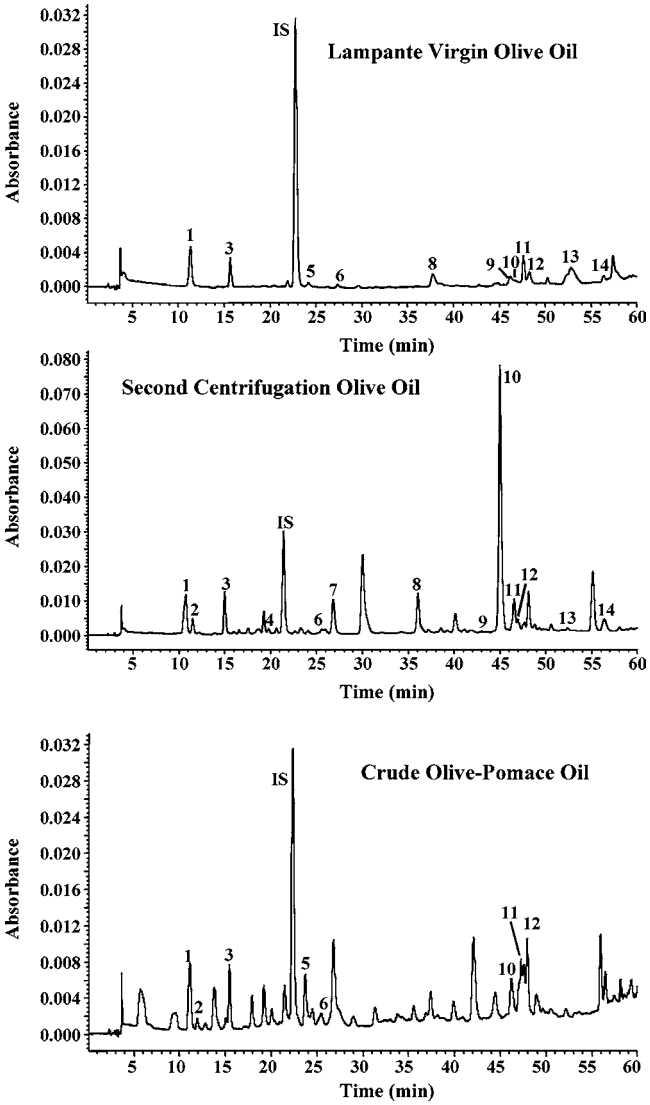

Figure 2. UV chromatogram at 280 nm of the phenolic extracts of different types of olive oils. Peaks: (1) hydroxytyrosol; (2) catechol; (3) tyrosol; (4) vanillic acid; (IS) internal standard, syringic acid; (5) vanillin; (6) Hy-AC, hydroxytyrosol acetate; (7) *p*-coumaric acid; (8) Hy-EDA, dialdehydic form of decarboxymethyl oleuropein aglycon; (9) Ty-EDA, dialdehydic form of decarboxymethyl ligstroside aglycon; (10) 4-ethylphenol; (11) 1-acetoxypinoresinol; (12) pinoresinol; (13) Hy-EA, oleuropein aglycon; (14) Ty-EA, ligstroside aglycon.

HPLC coupled to a mass spectrometer detector. In particular, the phenolic composition of lampante olive oils was almost similar to that of virgin olive oils (*9*, *10*), comprising the secoiridoid aglycons (Hy-EDA, Ty-EDA, Hy-EA, and Ty-EA), the lignans 1-acetoxypinoresinol and pinoresinol, the flavonoids luteolin and apigenin, and the simple phenols hydroxytyrosol, tyrosol, vanillin, and vanillic acid. At the same time, the presence of catechol in these LOO, as well as in COPO and SCOO, was detected and confirmed by retention time, UV, and mass spectra. This polyphenol has not been detected in olive oils (*8*, *9*, *11*), except in those obtained from fermented olives (*14*).

Another new peak identified in these oils was peak 10. Although it was found in all oils, its response under fluorescent excitation was particularly significant in SCOO. The phenolic extracts had to be diluted more than 1:100 to avoid saturation of the fluorescence detector. Its UV response was also significant, and the compound showed a UV spectrum similar to that of tyrosol with two absorbing bands at 221 and 277 nm.

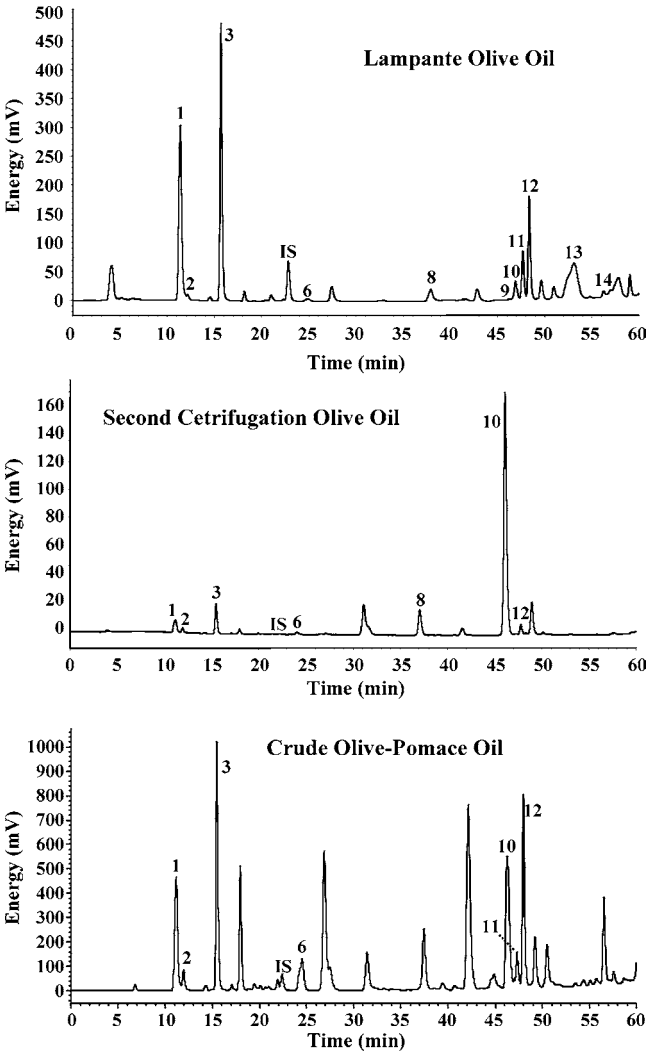

Figure 3. Fluorescence chromatogram of the phenolic extracts of different types of olive oils. Phenolic extract of the SCOO was diluted 1:100 with *N*,*N*-dimethylformamide. See legend of **Figure 2** for peak identification.

Surprisingly, the electrospray mass spectrometer technique was not able to monitor the compound under negative or positive ion modes, as was achieved for the rest of the olive polyphenols. Consequently, the compound was isolated by analytical HPLC and, because of its high volatility, it was not evaporated under vacuum in a rotatory evaporator but extracted with chloroform. This substance was first tentatively identified by GC-MS, and then its structure was confirmed by comparison of both its gas chromatographic retention time and its mass spectrum (**Figure 4**) with a genuine sample of 4-ethylphenol. The mass spectrum exhibited the molecular ion (33%), and the base peak was a loss of a methyl group. Other peaks were the *m*/*z* 91 (5%) and *m*/*z* 77 (16%) ions.

To our knowledge, there are three papers describing the presence of 4-ethylphenol in olive products. In particular, this compound was detected in small amounts in olive-mill wastewaters (*15*) and in the volatile fraction of VOO (*16*, *17*). However, it was not found in off-flavor oils (*18*).

4-Ethylphenol is a volatile phenol with an unpleasant odor of "wet horse" or "phenolic". We collected different fractions of mobile phase during chromatographic runs and sniffed them. The fraction corresponding to peak 10 (4-ethylphenol) had a strong off-odor similar to that of the phenolic extracts and crude oils, whereas any other collected fraction showed the same odor.

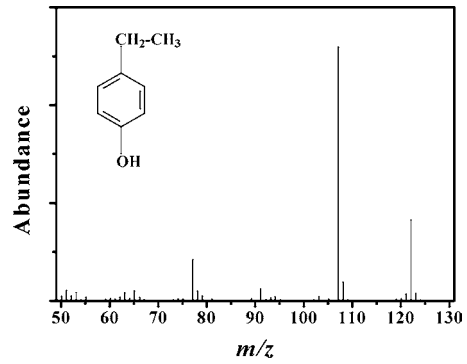

Figure 4. Mass spectrum of 4-ethylphenol.

Table 1. Content in Phenolic Compounds of Nine Different Lampante Olive Oils[a]

	olive oil								
compound[b]	A	B	C	D	E	F	G	H	I
hydroxytyrosol	20.5	60.8	127.6	17.8	2.2	0.6	1.1	3.8	2.6
catechol	nd[c]	2.3	1.4	0.9	4.1	0.4	3.1	15.6	53.5
tyrosol	30.9	13.5	38.5	12.1	3.0	2.5	6.5	2.4	4.0
vanillic acid	nd	nd	nd	nd	nd	nd	nd	0.5	2.9
vanillin	nd	0.4	nd	0.4	nd	nd	nd	0.3	nd
Hy-AC	nd	1.6	1.0	1.7	2.3	13.7	2.9	3.0	5.6
Hy-EDA	nd	35.9	36.1	22.8	nd	nd	8.0	9.3	137.6
Ty-EDA	nd	nd	18.6	33.0	nd	nd	8.8	14.3	1.7
4-ethylphenol	0.4	0.7	0.5	0.4	1.5	19.6	6.0	2.9	10.1
1-acetoxypinoresinol	10.7	37.4	2.1	26.8	8.5	2.6	nd	14.4	21.4
pinoresinol	29.2	19.5	37.4	33.3	13.7	22.0	16.2	11.6	9.6
Hy-EA	87.8	102.9	444.5	64.6	14.6	nd	2.1	4.0	14.6
Ty-EA	52.2	42.0	144.7	41.1	7.3	nd	nd	2.3	nd
luteolin	5.7	6.5	2.9	6.2	3.1	nd	2.8	4.2	7.1
apigenin	1.7	2.5	0.8	2.5	1.3	1.9	1.5	2.3	4.6

[a] Concentrations are expressed in mg/kg of oil and are means of two determinations. [b] Hy-AC, hydroxytyrosol acetate; Hy-EDA, dialdehydic form of decarboxymethyl oleuropein aglycon; Ty-EDA, dialdehydic form of decarboxymethyl ligstroside aglycon; Hy-EA, oleuropein aglycon; Ty-EA, ligstroside aglycon. [c] Compound not detected.

Therefore, it was concluded that the characteristic off-odor of these oils, in particular SCOO, was consistent with 4-ethylphenol.

4-Ethylphenol has been reported as occurring in various sources; for example, it is a component of the volatile fraction of cigarette smoke (*19*), and it contributes to the smoky taste of cocoa powder (*20*) and the bad smell of piggeries (*21*). It is generally formed by microorganisms, as, for example, in the intestinal tract of humans from the isoflavone genistein (*22*) or in red wines by the microorganism *Dekkera bruxellensis*, among other yeast species (*23*, *24*).

4-Ethylphenol was present in most LOO up to a concentration of 19.6 mg/kg of oil (**Table 1**) and in COPO up to 52 mg/kg of oil (**Table 2**). However, the richest source of this compound was the SCOO, in particular oils obtained from olive paste stored for 8 months (**Table 3**). It seems that this compound was formed during olive paste storage and reached up to 476 mg/kg of oil after 8 months of paste storage. We did not study any microorganism growth in olive paste during storage, but other researchers have reported the presence of bacteria (*25*) and yeasts (*26*) in this matrix, which could be responsible for the 4-ethylphenol formation.

SCOO was also enriched in other phenolic compunds during olive paste storage (**Table 3**). Thus, hydroxytyrosol ranged from 42 to 608 mg/kg in oils obtained from pastes stored for 3 and

Table 2. Content in Phenolic Compounds of Two Crude Olive Pomace Oils[a]

compound	olive pomace oil A	olive pomace oil B
hydroxytyrosol	115.1	93.6
catechol	30.2	17.1
tyrosol	48.4	29.2
vanillin	9.9	4.7
Hy-AC[b]	189.4	70.7
4-ethylphenol	52.2	15.0
1-acetoxypinoresinol	9.7	80.7
pinoresinol	161.7	88.6

[a] Olive paste was stored for 2 months prior to chemical extraction of oil with hexane. Concentrations are expressed in mg/kg and are the means of two determinations. [b] Hydroxytyrosol acetate.

Table 3. Content in Phenolic Compounds of Nine Different Second Centrifugation Olive Oils[a]

	olive oil								
	3 months of storage				8 months of storage				
compound[b]	A	B	C	D	A	B	C	D	E
hydroxytyrosol	47.7	58.5	42.7	63.5	488.5	149.2	540.1	346.7	608.5
catechol	7.1	4.4	6.8	6.3	176.1	51.9	173.7	97.5	172.4
tyrosol	19.5	24.4	34.1	21.8	156.6	60.9	85.2	50.5	83.6
vanillic acid	nd[c]	1.3	1.8	nd	7.6	9.5	13.1	9.2	nd
vanillin	nd	0.9	0.3	1.1	nd	nd	nd	nd	nd
Hy-AC	18.2	16.3	17.3	3.5	463.7	82.2	181.8	145.0	418.4
p-coumaric acid	nd	11.6	12.6	5.3	nd	13.9	nd	nd	nd
Hy-EDA	nd	56.0	20.6	60.7	nd	nd	nd	nd	nd
Ty-EDA	nd	nd	18.6	26.4	nd	nd	nd	nd	nd
4-ethylphenol	16.1	32.6	38.3	15.4	476.8	354.2	120.5	262.2	416.3
1-acetoxypino-resinol	41.7	11.3	47.1	10.3	158.3	38.4	128.9	54.2	72.7
pinoresinol	54.3	83.9	67.1	71.2	nd	nd	nd	nd	nd
Hy-EA	33.5	64.5	37.6	119.4	nd	15.9	nd	35.7	45.0
Ty-EA	17.8	28.9	17.5	59.0	nd	19.3	nd	nd	nd
luteolin	7.6	nd	5.5	4.3	8.5	9.1	10.9	9.6	9.8
apigenin	2.2	nd	1.9	0.9	4.9	3.4	4.7	5.4	3.5

[a] Concentrations are expressed in mg/kg of oil and the means of two determinations. [b] Hy-AC, hydroxytyrosol acetate; Hy-EDA, dialdehydic form of decarboxymethyl oleuropein aglycon; Ty-EDA, dialdehydic form of decarboxymethyl ligstroside aglycon; Hy-EA, oleuropein aglycon; Ty-EA, ligstroside aglycon. [c] Compound not detected.

8 months, respectively. This antioxidant is the most important polyphenol in olive oils, and it is present in VOO as a simple monomeric phenol, in a concentration <20 mg/kg of oil, or combined with elenolic acid and derivatives, in a concentration <250 mg/kg of oil (*27*). The latter secoiridoid aglycons almost disappeared in oils obtained from pastes stored for 8 months and were present in a concentration <120 mg/kg in oils from pastes stored for 3 months. Consequently, this means that the high amount of hydroxytyrosol found in oils from pastes stored for 8 months had not entirely originated from the hydrolysis of the secoiridoid aglycons and its diffusion from the non-lipid phases of olive paste to the oil. Likewise, the same deductions can be made for tyrosol and derivatives.

The orthodiphenol catechol and hydroxytyrosol acetate have never been found in such high concentrations in olive oils as found in SCOO from pastes stored for 8 months. The concentration of catechol reached up to 176 mg/kg of oil, which is much higher than that observed in the oil phase of table olives (*14*). Also, hydroxytyrosol acetate has never been reported to exceed a concentration of 250 mg/kg in VOO (*27*), even in oils of the Arbequina cultivar, which are particularly rich in this substance (*28*).

The behavior of the lignans was very surprising. SCOO gained 1-acetoxypinoresinol and lost pinoresinol during olive paste storage without any known explanation.

Other minor monomeric phenols that increased their concentration with olive paste storage were the flavonoids luteolin and apigenin and vanillic acid.

In contrast to the high concentration in polyphenols of SCOO, the COPO (extracted with solvent) had a very low amount of these substances (**Table 2**), hydroxytyrosol, its acetate, and lignans being the most representative. In addition, the HPLC-MS analyses of the phenolic extracts did not detect secoiridoid aglycons in these oils.

Finally, the high variability of polyphenol concentration found in VOO (*27*) was also observed in LOO (**Table 1**), although the latter oils had generally lower amounts of these substances than the former.

In conclusion, crude olive oils intended for refining contain a significant concentration of phenolic compounds, which, in the case of SCOO, is high enough to make their recovery attractive. On the basis of literature data (*12*), it seems that polyphenols are lost during refining, but their extraction during classical or new refining methods should be explored, and therefore this type of research is in progress.

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LITERATURE CITED

(1) Alba, J.; Hidalgo, F.; Ruiz, M. A.; Martínez, F.; Moyano, M. J.; Cert, A.; Pérez, M. C.; Ruiz, M. V. Characteristics of the olive oil obtained with first and second centrifugation. *Grasas Aceites* **1996**, *47*, 163−181.

(2) Regulation 1513/2001. *Off. J. Eur. Communities* **2001**.

(3) Regulation 1989/2003. *Off. J. Eur. Communities* **2003**.

(4) Ruiz-Méndez, M. V.; Dobarganes, M. C. Olive oil and olive pomace oil refining. *Oleagineux Corps Gras Lipides* **1999**, *6*, 56−60.

(5) Gutiérrez, F.; Albi, M. A.; Palma, R.; Ríos, J. J.; Olías, J. M. Bitter taste of virgin olive oil: correlation of sensory evaluation and instrumental HPLC analysis. *J. Food Sci.* **1989**, *54*, 68−70.

(6) Baldioli, M.; Servili, M.; Perretti, G.; Montedoro, G. F. Antioxidant activity of tocopherol and phenolic compounds of virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1589−1593.

(7) Visioli, F.; Galli, C.; Galli, G.; Caruso, D. Biological activities and metabolic fate of olive oil phenols. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 677−684.

(8) Montedoro, G. F.; Servili, M.; Baldioli, M.; Selvaggini, R.; Miniati, E.; Macchioni, A. Simple and hydrolyzable compounds in virgin olive oil. 3. Spectroscopic characterization of the secoiridoid derivatives. *J. Agric. Food Chem.* **1993**, *41*, 2228−2234.

(9) Brenes, M.; García, A.; García, P.; Ríos, J. J.; Garrido, A. Phenolic compounds in Spanish virgin olive oils. *J. Agric. Food Chem.* **1999**, *47*, 3535−3540.

(10) Brenes, M.; Hidalgo, F. J.; García, A.; Ríos, J. J.; García, P.; Zamora, R.; Garrido, A. Pinoresinol and 1-acetoxypinoresinol, two new phenolic compounds identified in olive oil. *J. Am. Oil Chem. Soc.* **2000**, *77*, 715−720.

(11) Zunin, P.; Evangelisti, F.; Pagano, M. A.; Tiscornia, E.; Petachi, R. Phenolic compounds in oil obtained from *Olea europaea* and anti-*Dacus* treatments. *Riv. Ital. Sostanze Grasse* **1995**, *72*, 55−59.

(12) Nergiz, C. The effect of refining process on the total polyphenol and 1,2-diphenol content of olive oil. *Int. J. Food Sci. Technol.* **1993**, *28*, 461−464.

(13) Brenes, M.; García, A.; Rios, J. J.; García, P.; Garrido, A. Use of 1-acetoxypinoresinol to authenticate Picual olive oils. *Int. J. Food Sci. Technol*. **2002**, *37*, 615−625.
(14) Romero, C.; Brenes, M.; Khaled, Y.; García, P.; García, A.; Garrido, A. Effect of cultivar and processing method on the contents of polyphenols in table olives. *J. Agric. Food Chem*. **2004**, *52*, 479−484.
(15) Camurati, F.; Lanzani, A.; Arpino, A.; Ruffo, C.; Fedeli, E. Waste waters from the olive-processing in the production of olive oils. Technologies and economical aspects in the recovery of by-products. *Riv. Ital. Sostanze Grasse* **1984**, *61*, 283−292.
(16) Cavalli, J. F.; Fernández, X.; Lizzani-Cuvelier, L.; Loiseau, A. M. Comparison of static headspace, headspace solid-phase microextraction, headspace sorptive extraction, and direct thermal desorption techniques on chemical compositon of French olive oils. *J. Agric. Food Chem*. **2003**, *51*, 7709−7716.
(17) Lee, J. H.; Kim, M. R.; Kim, I. H.; Kim, H.; Shin, J. A.; Lee, K. T. Physicochemical and volatile characterization of structured lipids from olive oil produced in a stirred-tank batch reactor. *J. Food Sci*. **2004**, *69*, 89−95.
(18) Sánchez-Saez, J. J.; Herce-Garraleta, M. D.; Balea-Otero, T. Identification of cinnamic acid ethyl ester and 4-vinylphenol in off-flavour olive oils. *Anal. Chim. Acta* **1991**, *247*, 295−297.
(19) Clark, T. J.; Bunch, J. E. Quantitative determination of phenols in mainstream smoke with solid-phase microextraction-gas chromatographic-selected ion monitoring mass spectrometry. *J. Chromatogr. Sci.* **1996**, *34*, 272−275.
(20) Bonvehi, J. S.; Coll, F. V. Evaluation of smoky taste in cocoa powder. *J. Agric. Food Chem*. **1998**, *46*, 620−624.
(21) Spoelstra, S. F. Simple phenols and indoles in anaerobically stored piggery wastes. *J. Sci. Food Agric.* **1977**, *28*, 415−423.
(22) Bathena, S. J.; Velasquez, M. T. Beneficial role of dietary phytoestrogens in obesity and diabetes. *Am. J. Clin. Nutr*. **2002**, *76*, 1191−1201.
(23) Días, L.; Días, S.; Sancho, T.; Stender, H.; Querol, A.; Malfeito-Ferreira, M.; Loureiro, V. Identification of yeasts isolated from wine-related environments and capable of producing 4-ethylphenol. *Food Microbiol*. **2003**, *20*, 567−574.
(24) Chatonnet, P.; Viala, C.; Dubourdieu, D. Influence of polyphenolic components of red wines on the microbial synthesis of volatile phenols. *Am. J. Enol. Vitic*. **1997**, *48*, 443−448.
(25) Jones, C. E.; Murphy, P. J.; Russell, N. J. Diversity and osmoregulatory responses of bacteria isolated from two-phase olive oil extraction waste products. *World J. Microbiol. Biotechnol*. **2000**, *16*, 555−561.
(26) Giannoutsou, E. P.; Meintanis, C.; Karagouni, A. D. Identification of yeast strains isolated from a two-phase system olive oil waste and investigation of their ability of its fermentation. *Bioresour. Technol*. **2004**, *93*, 301−306.
(27) García, A.; Brenes, M.; García, P.; Romero, C.; Garrido, A. Phenolic content of commercial olive oils. *Eur. Food Res. Technol.* **2003**, *216*, 520−525.
(28) Romero, M. P.; Tovar, M. J.; Girona, J.; Motilva, M. J. Changes in the HPLC phenolic profile of virgin olive oil from young trees (*Olea europaea* L. Cv. Arbequina) grown under different deficit irrigation strategies. *J. Agric. Food Chem.* **2002**, *50*, 5349−5354.

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