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Hydroxytyrosol 4-β-D-Glucoside, an Important Phenolic Compound in Olive Fruits and Derived Products

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There is increasing interest in olive polyphenols because of their biological properties as well as their contribution to the color, taste, and shelf life of olive products. However, some of these compounds remain unidentified. It has been shown that hydroxytyrosol 4-β-D-glucoside (4-β-D-glucosyl-3-hydroxyphenylethanol) coeluted with hydroxytyrosol [(3,4-dihydroxyphenyl)ethanol] under reversed phase conditions in the phenolic chromatograms of olive pulp, vegetation water, and pomace of olive oil processing. A method to separate this compound from hydroxytyrosol by HPLC has been developed. The concentration of this glucoside increased in olive pulp with maturation and could be the main phenolic compound in mature olives. In contrast, the presence of this compound was not detected in olive oil by using HPLC-MS. The compound must be considered both in table olives and olive oil processing because of its glucose and hydroxytyrosol contribution to these products.

KEYWORDS: Hydroxytyrosol; hydroxytyrosol 4-β-D-glucoside; olives; phenolic compounds

INTRODUCTION

Hydroxytyrosol [(3,4-dihydroxyphenyl)ethanol] is one of the major natural phenolic compounds present in olive fruits, virgin olive oil, table olives, and waste streams generated during olive processing (*1*−*4*). This compound has shown antimicrobial, hypoglycemic, hypolipidemic, and hypocholesterol properties of particular interest with regard to food and human health (*5*−*7*).

This substance can be found in olive products either as the simple phenol or esterified with elenolic acid to form oleuropein and its aglycon (*1*−*3*) and as a part of the verbascoside molecule (*1*). Furthermore, it has also been found as a glucoside in olive pulp and virgin olive oil (*8*−*10*) as well as in green pepper berries (*11*). Bianco et al. (*9*) identified three different hydroxytyrosol glucosides in olives and virgin olive oil depending on the hydroxyl group to which the glucose molecule was bound. Bianco and Uccella (*12*) analyzed phenolic compounds in olives and found hydroxytyrosol glucosides; however, their peaks were not shown in an HPLC chromatogram, and the amount of these substances existing in olive fruits was reported as not important.

In this investigation it is demonstrated that hydroxytyrosol 4-β-D-glucoside has not been observed previously in the HPLC chromatograms because of its coelution with hydroxytyrosol. A method of separating these two peaks and the quantification of these compounds in the different olive products and waste have also been developed.

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MATERIALS AND METHODS

Sample Preparation. Olive fruits of the Manzanilla variety with different ripeness indexes (ir = 1, 1.2, 1.8, 3, and 4), calculated as a subjective evaluation color of the skin and flesh proposed by Uceda and Frías (*13*), and olives of the Picual variety (ir = 3) were hand collected in the 2000 crop year. The olive fruits were frozen in liquid nitrogen and crushed in a mortar after removal of the stone, yielding a uniform powder.

Olive oil, vegetation water, and pomace were obtained from Picual olives by an Abencor analyzer (Comercial Abengoa, S.A., Seville, Spain) (*14*). Crushing of olives (570 g) was performed using an inox hammer mill operating at 3000 rpm and provided with a sieve with 5 mm holes. Then, the malaxation step was carried out in a mixer at 14 rpm and 30 °C for 35 min, adding a total of 300 mL of boiling water. Finally, three phases (oil, vegetation water, and pomace) were separated by centrifugation (8 min, 7000 rpm) and the oil phase was filtered by gravity.

Analysis of Phenolic Compounds. *Phenolic Extraction.* The phenolic extracts of olive fruits and pomace were obtained following the procedure described in **Figure 1** and based on that of Servili et al. (*3*). The proposed method consisted of extracting the phenolic compounds with a solution of methanol/water plus 100 ppm of sodium salt of diethyldithiocarbamic acid. A C_{18} cartridge was used to purify the phenolic extract, and syringic acid was used as internal standard.

The phenolic extract of olive oil was obtained following the procedure proposed by Brenes et al. (*15*). The vegetation water was filtered through 0.45 μm filter and injected into the chromatograph.

HPLC Analysis of Phenolic Compounds. Two HPLC systems were used.

Chromatography System 1. The HPLC system consisted of a Waters 2690 Alliance with a pump, column heater, and autosampler modules included, the detection being carried out with a Waters 996 photodiode array detector. The system was controlled with Millennium[32] software

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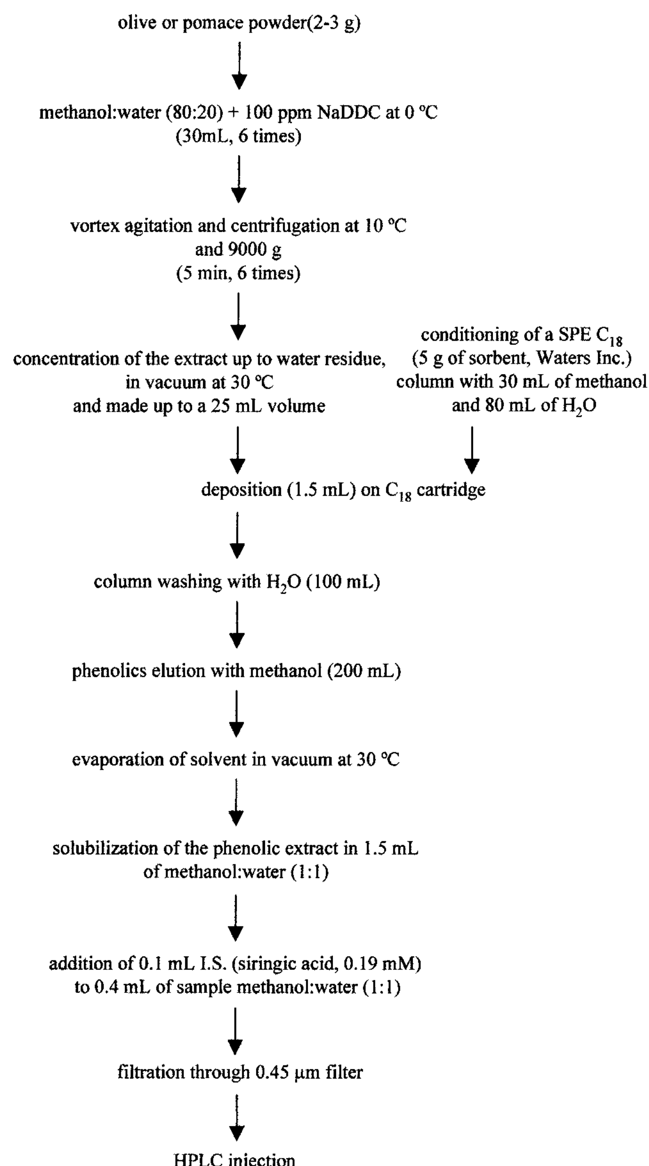

Figure 1. Flowchart of extraction and separation of phenolic compounds from olives.

(Waters Inc., Milford, MA). A 25 cm × 4.6 mm i.d., 5 μm, Lichrospher 100 (Merck, Darmstadt, Germany) column was used for routine analyses, and 25 cm × 4.6 mm i.d., 5 μm, Ultrasphere ODS (Beckman, Fullerton, CA), 25 cm × 4.6 mm i.d., 5 μm, Spherisorb ODS-2 (Waters Inc.), and 25 cm × 4.6 mm i.d., 5 μm, Extrasil ODS-2 (Technokroma, Barcelona, Spain) columns were used in special cases. Separation was achieved by gradient elution using an initial composition of 90% water (pH 2.5 adjusted with 0.15% phosphoric acid) and 10% methanol. The concentration of the latter solvent was increased to 30% in 10 min and maintained for 20 min. Subsequently, the methanol percentage was raised to 40% in 10 min, which was maintained for 5 min. Finally, the methanol percentage for the last three steps was increased to 60, 70, and 100% in 5 min periods. Initial conditions were reached in 15 min. An injection volume of 20 μL, a flow rate of 1 mL/min, and a temperature of 35 °C were used. Chromatograms were recorded at 280 nm.

Chromatography System 2. The HPLC system consisted of a Waters 717 plus autosampler, a Waters 600 E pump, a Waters column heater module, and a Waters 996 photodiode array detector operated with Millenium 2010 software (Waters Inc.). A 25 cm × 4.6 mm i.d., 5 μm, Lichrospher 100 (Merck) column was used. Separation was carried out under conditions similar to those given above under Chromatography System 1.

Table 1. Response Factor (f_x) and Determination Coefficient (r^2) Corresponding to the Calibration Curves[a] of Reference Compounds (S)[b]

compound	f_x	r^2
hydroxytyrosol 4-β-D-glucoside	0.082	0.996
hydroxytyrosol	0.170	0.993
tyrosol	0.129	0.999
luteolin 7-*O*-glucoside	1.032	0.998
rutin	0.246	0.997
verbascoside	29.61	0.997
oleuropein	0.224	0.993

[a] Area (S)/area(I.S.) = f_x [concentration (S)/concentration (I.S.)] [b] Syringic acid (0.19 mM) was used as internal standard (I.S.). Detection was made at 280 nm, and samples were diluted 4:1 (S/I.S.).

Reference Compounds. The evaluation of each compound was performed using a regression curve in triplicate of three points (**Table 1**). Oleuropein and luteolin 7-*O*-glucoside were purchased from Extrasynthese S.A. (Lyon Nord, Genay, France); tyrosol and rutin were provided by Sigma Chemical Co. (St. Louis, MO). Verbascoside was isolated by analytical HPLC, and its absorbance was measured at 330 nm in a 10 mm quartz cell; the concentration was calculated using the molar absorptivity value of 111 (*16*). Hydroxytyrosol and hydroxytyrosol 4-β-D-glucoside were obtained using a 25 cm × 20 mm i.d., 5 μm, Spherisorb ODS-2 preparative HPLC column (Waters Inc.) and a flow rate of 16 mL/min. The HPLC system consisted of a Waters 600 E pump, a Rheodyne 7125 injector with a 1 mL loop, a Waters fraction collector II, and a Waters 994 photodiode array detector. The mobile phases were water and methanol. The initial condition of 20% methanol was maintained for 10 min, then increased linearly to 100% in 5 min, and maintained for 5 min to clean the column. The initial conditions were achieved in 5 min, and then equilibrated for another 10 min before a new injection. The fractions containing the recovered substances were pooled and evaporated under vacuum, and the residue was dissolved in a minimum amount of deionized water. Finally, the aqueous residue was frozen and freeze-dried (model Alpha 1-4; Martin Christ, Osterode am Harz, Germany).

HPLC-MS Analysis of Phenolic Compounds. Sample extracts were analyzed using a ZMD4 mass spectrophotometer (Waters Inc.) equipped with an electrospray ionization ion source (ESI). The ionspray mass spectra in the negative-ion mode were obtained under the following conditions: capillary voltage, 3 kV; cone voltage, 20 V; extractor voltage, 12 V; desolvation temperature, 250 °C; and source temperature, 80 °C. A constant flow of 1 mL/min was used for each analysis, with an approximately 4:1 split ratio (UV detector-MS).

NMR Analyses. ^{1}H and ^{13}C NMR spectra, at 300 and 75.4 MHz, respectively, were determined on a Bruker AC-300P instrument (Karlsruhe, Germany) using tetramethylsilane as an internal standard. Hydroxytyrosol 4-β-D-glucoside: ^{13}C NMR δ 146.44 (C-4), 143.99 (C-3), 136.04 (C-1), 122.15 (C-2), 117.89 (C-6), 117.74 (C-5), 102.25 (C-1′), 77.02 (C-5′), 76.36 (C-3′), 73.76 (C-2′), 70.21 (C-4′), 63.38 (C-1β), 61.31 (C-6′), 38.02 (C-1α).

RESULTS AND DISCUSSION

A peak (peak 1) close to that of hydroxytyrosol (peak 2) was observed in all of the phenolic chromatograms of pulp, vegetation water, and pomace of Picual olives (**Figure 2**). To our knowledge, this is the first time that a peak eluting close to that of hydroxytyrosol has been detected in the phenolic chromatograms of olives and derived products (*1*−*3*). We first assumed that it could be a precolumn fouling problem, but this was ruled out because double peaks were also detected with a new precolumn. The UV spectra of the two compounds were very similar. Electrochemical detection was also studied (*15*) to distinguish between these two peaks, and the responses of these compounds to several applied voltages (250, 500, 750, and 1000 mV) were very different, the dominant potential being

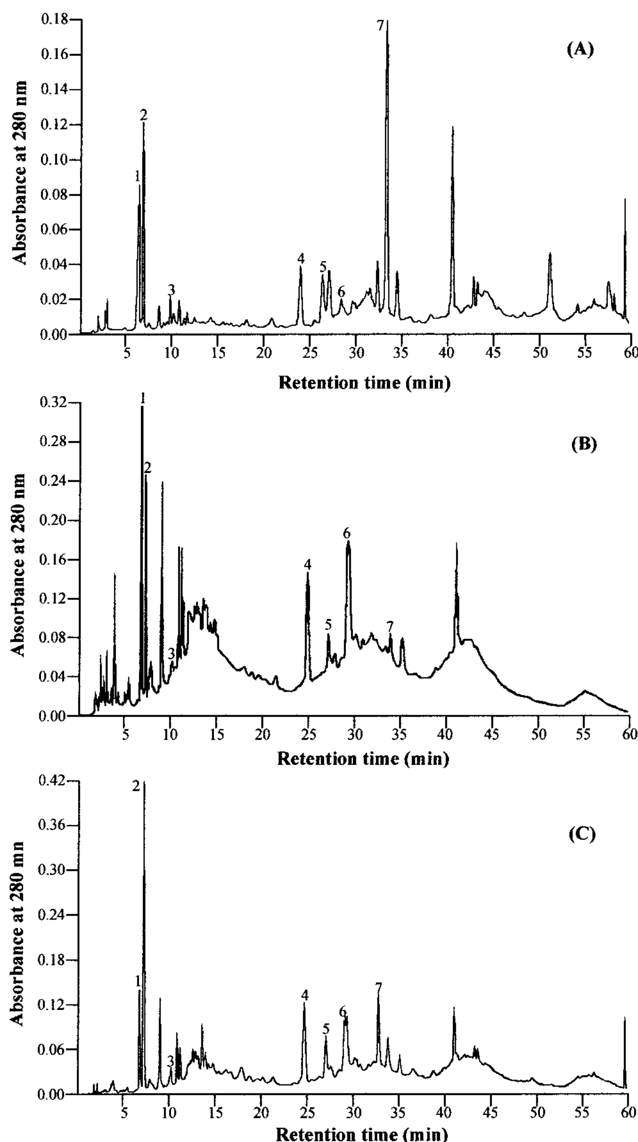

Figure 2. HPLC chromatograms of phenolic compounds in olive pulp (A), vegetation water (B), and pomace (C). Samples were obtained from Picual olives. Peaks: (1, 2) hydroxytyrosol species; (3) tyrosol; (4) verbascoside; (5) luteolin 7-*O*-glucoside; (6) rutin; (7) oleuropein.

250 and 500 mv for hydroxytyrosol and peak 1, respectively. The dominant potential for *o*-diphenols is 250 mV (*15*) as occurred for hydroxytyrosol and, therefore, the compound corresponding to peak 1 must not have hydroxyl groups at the ortho position.

The new compound was detected in olive pulp, vegetation water, and pomace, but its presence had not previously been reported in olive phenolic chromatograms by other research groups. One possibility could be the type of analytical column used for the separation of the phenolic compounds. We tried to separate peak 1 from that of hydroxytyrosol by using C_{18} reversed phase columns of four different brands (**Figure 3**). Good resolution was achieved with Lichrospher 100 and Ultrasphere ODS columns, poor resolution with a Spherisorb ODS-2 column, and no separation of the two peaks when using an Extrasil ODS-2 column. This could therefore explain why the presence of peak 1 in phenolic chromatograms has not been reported until now. Another possibility could be the chromatographic system employed because the resolution of peaks also depends on this factor. With the same Lichrospher 100 column

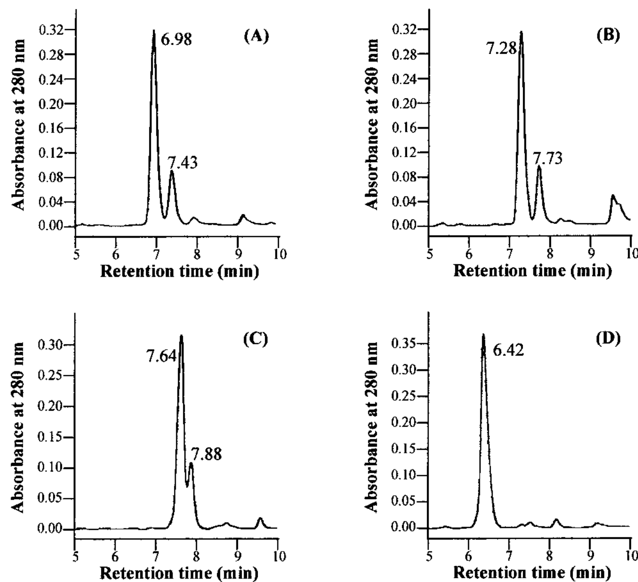

Figure 3. Separation of peaks 1 and 2 on reversed phase C_{18} columns: (A) Lichrospher 100; (B) Ultrasphere ODS; (C) Spherisorb ODS-2; (D) Extrasil ODS-2.

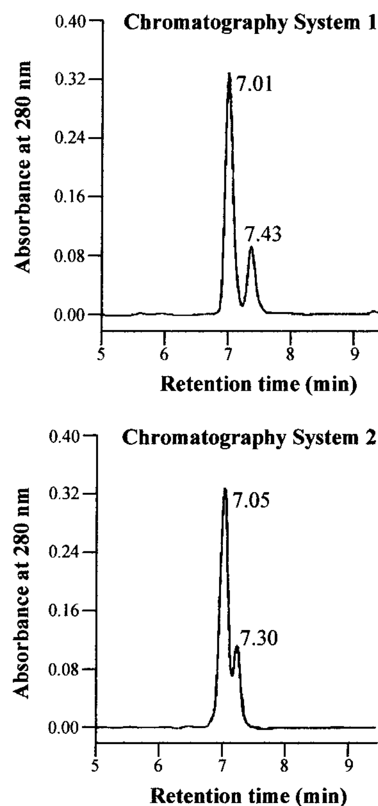

Figure 4. Separation of peaks 1 and 2 on Lichrospher 100 column using two different chromatography systems (see Materials and Methods for details).

and the same gradient and eluents, a different separation of peak 1 and hydroxytyrosol was obtained when the analysis was run under two different chromatographic systems (**Figure 4**). A better resolution of peaks 1 and 2 was obtained when chromatographic system 1 was used rather than system 2, which must be related to the lower dwell volume in system 1 compared to system 2.

A further reason peak 1 has not been detected until now may be the extraction procedure. Many researchers have extracted

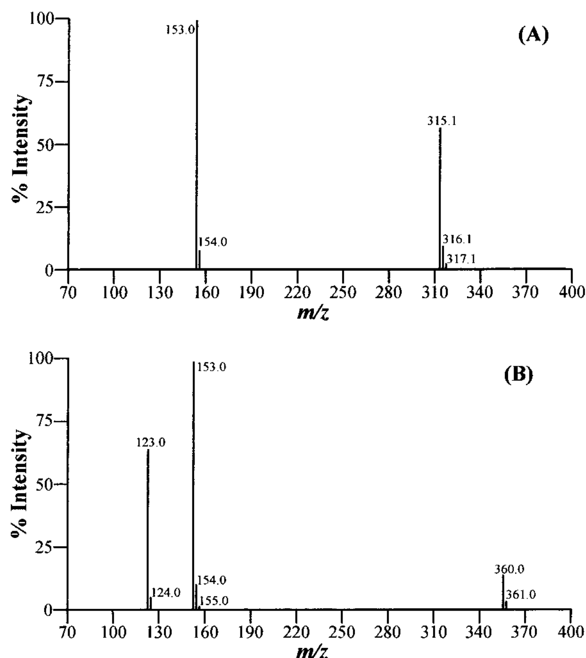

Figure 5. Negative ion mass spectra (ESI) of hydroxytyrosol 4-β-D-glucoside (A) and hydroxytyrosol (B) under cone voltage of 20 V.

polyphenols from olives with a water/alcohol mixture (ethanol or methanol), evaporated the organic solvent, and extracted the polyphenols from the aqueous residue with ethyl acetate (*1*, 2). It was found that ethyl acetate did not extract peak 1, which remained in the aqueous residue (data not shown), because this compound is much more polar than hydroxytyrosol.

Peak 1 has not been reported in Spanish-style green olive brines or black ripe olive brines (*2*, *17*) when analyzed by direct injection of the centrifuged brines in the chromatograph. In these cases, it may be supposed that an acid or alkaline hydrolysis of compound **1** took place during processing, as occurs for the glucoside oleuropein (*2*). The main phenolic compounds identified in olives treated with NaOH were hydroxytyrosol, tyrosol, caffeic acid, and *p*-coumaric acid. Therefore, if peak 1 was hydrolyzed during the alkaline treatment, it must be made up in part of one of the four phenolic compounds noted above.

Peak 1 was isolated by preparative HPLC, and the complete identification of this compound was carried out by NMR and MS. The mass spectrum of this compound displayed major signals at *m*/*z* 315 and 153 (**Figure 5**). Thus, the fragment at 153 could correspond to hydroxytyrosol, which could be bound to glucose, explaining in turn the signal at 315 as the molecular ion. In fact, a significant fragment at *m*/*z* 123 corresponding to hydroxytyrosol was also detected for peak 1 when the mass spectrometer was operated at a cone voltage of 70 V (data not shown). Therefore, on the basis of the MS data obtained, it may be assumed that peak 1 is formed from hydroxytyrosol and glucose. The glycosidic bond must be located on one of the hydroxyl groups of the aryl ring as this compound did not show the same response as hydroxytyrosol when electrochemical detection was used. Sugiyama and Kikuchi (*18*) reported the ^{13}C NMR data for hydroxytyrosol 3-β-D-glucoside, and Bianco et al. (*9*) showed the corresponding data of hydroxytyrosol 4-β-D-glucoside; our ^{13}C NMR data agree with these reported by Bianco et al. (*9*), and, in conclusion, our peak 1 could be assigned to hydroxytyrosol 4-β-D-glucoside (**Figure 6**). These researchers detected the presence of this compound and the other hydroxytyrosol glucosides in fruits and olive oil (*12*). However, the presence of hydroxytyrosol 4-β-D-glucoside in the phenolic

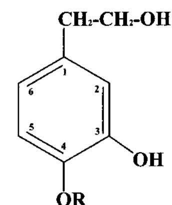

Figure 6. Chemical structure of hydroxytyrosol (R = H) and hydroxytyrosol 4-β-D-glucoside (R = 4-β-D-glucose).

chromatograms of olives was not shown and, the importance of this substance in olives and derived products has not been recognized (*10*, *12*).

In our experiments we detected the presence of hydroxytyrosol 4-β-D-glucoside in olive pulp of Manzanilla and Picual varieties. Occasionally, it has also been detected in other olive cultivars (data not shown). Those varieties were chosen because Manzanilla and Picual olives are the two most frequently used varieties for producing table olives and olive oil, respectively. Likewise, the presence of this hydroxytyrosol glucoside was detected for the first time in the vegetation waters and pomace of olive oil processing. This point must be stressed because extracts rich in hydroxytyrosol have been employed in some experiments considering biological properties of this compound, and this extract could be a mixture of hydroxytyrosol and hydroxytyrosol 4-β-D-glucoside (*19*, *20*).

Another interesting question is how much hydroxytyrosol 4-β-D-glucoside there is in olives. To determine this, the response factor of this compound with respect to an internal standard (syringic acid) was determined (**Table 1**), as were those of the other phenolic compounds in olives. It should be noted that the response factor of hydroxytyrosol 4-β-D-glucoside is different from that of hydroxytyrosol. Thus, if this compound were to be quantified with the response factor of hydroxytyrosol, an underestimation would be obtained.

As mentioned above, hydroxytyrosol 4-β-D-glucoside was detected in olive pulp, vegetation water, and pomace; however, we did not find this compound in virgin olive oil, as expected from previous work (*9*). Commercial and laboratory virgin olive oils were analyzed using HPLC-MS with an ESI probe in negative mode, a cone voltage of 20 V, and an extract ion at *m*/*z* 315. Hydroxytyrosol glucoside is a very polar compound and, therefore, it is reasonable that it should not be found in olive oil. In contrast, this compound was detected both in vegetation water and in pomace obtained on laboratory and industrial scales from the virgin olive oil processing. In the future, care should be taken in the quantitation of "hydroxytyrosol" obtained from these sources because it could be a mixture of the phenol and its glucoside (*4*, *21*).

It has been extensively reported (*1*) that oleuropein is the main phenolic compound in olive fruits and that its concentration decreases with maturation, although this depends on the olive variety and the interval ripeness index studied (*1*, *12*). In our experiments the concentration of this bitter glucoside did not show a clear tendency with maturation but, in contrast, the increase in the ripeness index of olives gave rise to an increase in the amount of hydroxytyrosol 4-β-D-glucoside in pulp (**Table 2**). In fact, the latter compound was second in importance in olive fruits, and with increased maturation it could become the most important. In the case of table olives hydroxytyrosol 4-β-D-glucoside must provide an important amount of the fermentable glucose in addition to that present in the free form in olive flesh (*22*). It should also be taken into account in the future that the amount of hydroxytyrosol in brines and fruits of table

Table 2. Phenolic Compounds (Millimoles per Kilogram of Dry Weight) in Manzanilla Olive Fruits with Different Ripeness Indexes ($n = 3$)

compound	ripeness index (ir)				
	1	1.2	1.8	3	4
hydroxytyrosol 4-β-D-glucoside	24.9 (3.8)[a]	26.8 (2.4)	29.9 (1.7)	48.4 (2.3)	64.1 (1.2)
hydroxytyrosol	12.2 (4.3)	9.3 (1.8)	8.1 (2.1)	7.8 (1.0)	8.5 (0.5)
tyrosol	1.8 (0.7)	1.2 (0.3)	0.8 (0.3)	1.0 (0.2)	0.8 (0.1)
luteolin 7-O-glucoside	0.8 (0.1)	0.9 (0.0)	0.7 (0.0)	1.1 (0.1)	1.2 (0.1)
rutin	0.5 (0.1)	0.6 (0.1)	0.5 (0.1)	0.8 (0.1)	0.9 (0.1)
verbascoside	0.2 (0.0)	0.1 (0.0)	0.8 (0.0)	0.1 (0.0)	0.2 (0.0)
oleuropein	70.3 (6.3)	84.3 (4.8)	75.6 (5.3)	99.1 (2.9)	89.6 (1.4)

[a] Standard deviation is given in parentheses.

olive processing comes not only from the hydrolysis of oleuropein (*23*) and verbascoside but also from the hydrolysis of hydroxytyrosol 4-β-D-glucoside.

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LITERATURE CITED

(1) Amiot, M. J.; Fleuriet, A.; Macheix, J. J. Importance and evolution of phenolic compounds in olive during growth and maturation. *J. Agric. Food Chem.* **1986**, *34*, 823−826.
(2) Brenes, M.; Rejano, L.; García, P.; Sánchez, A. H.; Garrido, A. Biochemical changes in phenolic compounds during Spanish-style green olive processing. *J. Agric. Food Chem.* **1995**, *43*, 2702−2706.
(3) Servili, M.; Baldioli, M.; Selvaggini, R.; Miniati, E.; Macchioni, A.; Montedoro, G. High-performance liquid chromatography evaluation of phenols in olive fruit, virgin olive oil, vegetation waters, and pomace and 1D- and 2D-nuclear magnetic resonance chatacterization. *J. Am. Oil Chem. Soc*. **1999**, *76*, 873−882.
(4) Mulinacci, N.; Romani, A.; Galardi, C.; Pinelli, P.; Giaccherini, C.; Vincieri, F. F. Polyphenolic content in olive oil waste waters and related olive samples. *J. Agric. Food Chem.* **2001**, *49*, 3509−3514.
(5) Ruiz, J. L.; Brenes, M.; Jiménez, R.; García, P.; Garrido, A. Inhibition of *Lactobacillus plantarum* by polyphenols extracted from two different kinds of olive brine. *J. Appl. Bacteriol*. **1993**, *74*, 15−19.
(6) Manna, C.; Della Ragione, F.; Cucciolla, V.; Borriello, A.; D'Angelo, S.; Galletti, P.; Zappia, V. Biological effects of hydroxytyrosol, a polyphenol from olive oil endowed with antioxidant activity. In *Advances in Nutrition and Cancer 2*; Zappia, V., Della Ragione, F., Barbarisi, A., Russo, A., Dello Iacovo, G. L., Eds.; Kluwer Academic/Plenum Publishers: New York, 1999; pp 115−130.
(7) Aruoma, O. I.; Deiana, M.; Jenner, A.; Halliwell, B.; Kaur, H.; Banni, S.; Corongiu, F.; Dessí, M. A.; Aeschbach, R. Effect of hydroxytyrosol found in extra virgin olive oil on oxidative DNA damage and on low-density lipoprotein oxidation. *J. Agric. Food Chem*. **1998**, *46*, 5181−5187.
(8) Vázquez, A.; Graciani, E.; Maestro, R. Componentes fenólicos de la aceituna. I. Polifenoles de la pulpa. *Grasas Aceites* **1974**, *25*, 269−279.
(9) Bianco, A.; Mazzei, R. A.; Melchioni, C.; Romeo, G.; Scarpati, M. L.; Soriero, A.; Uccella, N. Microcomponents of olive oil-III. Glucosides of 2(3,4-dihydroxyphenyl)ethanol. *Food Chem*. **1998**, *63*, 461−464.
(10) Ryan, D.; Lawrence, P.; Prenzler, P. D.; Antolovich, M.; Robards, K. Recovery of phenolic compounds from *Olea europaea*. *Anal. Chim. Acta* **2001**, *445*, 67−77.
(11) Variyar, S. P.; Pendharkar, M. B.; Banerjee, A.; Bandyopadhyay, C. Blackening in green pepper berries. *Phytochemistry* **1988**, *27*, 715−717.
(12) Bianco, A.; Uccella, N. Biophenolic components of olives. *Food Rev. Int*. **2000**, *33*, 475−485.
(13) Uceda, M.; Frías, L. Harvest dates. Evolution of the fruit of content, oil composition and oil quality. In *Proceedings of II Seminario Oleícola International*; International Olive-oil Council: Córdoba, Spain; 1975; pp 125−130.
(14) Martínez, J. M.; Muñoz, E.; Alba, J.; Lanzón, A. Report about the use of the "Abencor" yields analyzer. *Grasas Aceites* **1975**, *26*, 379−385.
(15) Brenes, M.; García, A.; García, P.; Garrido, A. Rapid and complete extraction of phenols from olive oil and determination by means of a coulometric electrode array system. *J. Agric. Food Chem*. **2000**, *48*, 5178−5183.
(16) Amiot, M. J.; Tachini, M.; Fleuriet, A.; Macheix, J. J. The technological debittering process of olives: Characterization of fruits before and during alkaline treatment. *Sci. Aliments* **1990**, *10*, 619−631.
(17) Brenes, M.; García, P.; Durán, M. C.; Garrido, A. Concentration of phenolic compounds change in storage brines of ripe olives. *J. Food Sci*. **1993**, *58*, 347−350.
(18) Sugiyama, M.; Kikuchi, M. Studies on the constituents of *Osmanthus Species* X. Structures of phenolic glucosides from the leaves of *Osmanthus asiaticus* Nakai. *Chem. Pharm. Bull.* **1992**, *40*, 325−326.
(19) Visioli, F.; Caruso, D.; Plasmati, E.; Patelli, R.; Mulinacci, N.; Romani, A.; Galli, G.; Galli, C. Hydroxytyrosol, as a component of olive mill waste water, is dose-dependently absorbed and increases the antioxidant capacity of rat plasma. *Free Radical Res*. **2001**, *34*, 301−305.
(20) Léger, C. L.; Kadiri-Hassani, N.; Descomps, B. Decreased superoxide anion production in cultured human promonocyte cells (THP-1) due to polyphenol mixtures from olive oil processing wastewaters. *J. Agric. Food Chem*. **2000**, *48*, 5061−5067.
(21) Fernández-Bolaño, J.; Felizón, B.; Brenes, M.; Guillén, R.; Heredia, A. Hydroxytyrosol and tyrosol as the main compounds found in the phenolic fraction of steam-exploded olive stones. *J. Am. Oil Chem. Soc*. **1998**, *75*, 1643−1649.
(22) García, P.; Brenes, M.; Romero, C.; Garrido, A. Respiration and physicochemical changes in harvested olive fruits. *J. Hortic. Sci*. **1995**, *70*, 925−933.
(23) Brenes, M.; Castro, A. Transformation of oleuropein and its hydrolysis products during Spanish-style green olive processing. *J. Sci. Food Agric*. **1998**, *77*, 353−358.

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