JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2008,** *56,* 5181–5187 **5181**

Correlations of the Phenolic Compounds and the Phenolic Content in Some Spanish and French Olive Oils

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The total content of phenolic compounds (TAP) in 29 different monocultivar olive oil samples from France (Aglandau and Tanche) and Spain (Cornicabra, Picual, and Verdial) was assessed by the colorimetric Folin−Ciocalteu method. Also, individual phenolic compounds were determined and quantified by liquid chromatography coupled to mass spectrometry (LC-MS). The French olive oil samples had a lower TAP compared to Spanish samples. The quantity of individual phenolics was similar except for pinoresinol, which was lower in the French olive oil samples. TAP moderately correlated to the sum of quantified compounds ($r = 0.64$ and $p < 0.01$) Partial least-squares (PLS) regression analysis emphasized the importance of hydroxytyrosol and the total amount of quantified phenolic compounds by LC-MS in the prediction of the total amount of phenolic compounds as determined by the Folin−Ciocalteu method. The amount of α-tocopherol was generally different among the cultivars (Tanche > Picual > Verdial > Aglandau > Cornicabra). Of all quantified phenolic compounds in French olive oil samples, only luteolin correlated well to the altitude of the olive orchards ($r = 0.76$, $p < 0.01$).

KEYWORDS: Olive oil; phenolic compounds; correlations; partial least-squares regression analysis

INTRODUCTION

Extra virgin olive oil (EVOO) contains a complex mixture of phenolic compounds. Among those mostly discussed are phenolic alcohols, that is, hydroxytyrosol (3,4-dihydroxyphenylethanol; 3,4-DHPEA) and tyrosol (*p*-hydroxyphenylethanol; *p*-HPEA), simple phenolic acids, flavonoids, lignans (acetoxypinoresinol and pinoresinol), oleosidic forms of hydroxytyrosol and tyrosol, and oleuropein aglycon (3,4-DHPEA-EA) and ligstroside aglycone (*p*-HPEA-EA) (*1*–*4*). Bendini et al. (*12*) stressed the need for further characterization of phenolic compounds in olive oil.

The issue of the content of phenolic compounds in olive oil being under many influences has been addressed in the literature (*5*–*11*). Although qualitative differences between the phenolic profiles in the different olive cultivars are less evident, there is a more pronounced quantitative variation among oils from different cultivars. Spanish and Italian cultivars have been extensively studied, whereas oils produced in other olive-growing regions have not frequently been addressed. Among different olive cultivars grown in France are Aglandau and Tanche, which result in oils of fruity taste and good storability. However, their phenolic profile is not very well-known, which can pose difficulties in the controlled designation or strict labeling of the oil according to cultivar type.

In addition to olive cultivar, the importance of harvest year has been demonstrated by relating the content of phenolic compounds and the quality of olive oils from five successive harvest years (*13*). The changes in content of phenolic compounds have also been monitored during different ripening stages of olives in one harvesting season (*14*). Moreover, cultivation conditions, such as different irrigation regimens (*15*), have an effect on olive oil quality and content of phenolic compounds. The altitude of the olive orchards may also influence the quality of olive oil, as shown by correlations established between altitude and fatty acid profile (*7*) or amount of sterols (*16*). However, to date, altitude has not directly been correlated to the amount and profile of phenolic compounds.

This study aimed to evaluate the content of phenolic compounds in several oil samples produced from Aglandau and Tanche olives. A comparison in and correlation between the

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10.1021/jf0724419 CCC: $40.75
Published on Web 06/13/2008

Table 1. Content of Phenolic Compounds (Milligrams per Kilogram) and α-Tocopherol (Milligrams per Kilogram) in Aglandau Olive Oil

compound	FR a1	FR a2	FR a3	FR a4	FR a5	av
3,4-DHPEA	24.06 ± 0.48a	14.22 ± 1.21b	14.83 ± 1.34b	17.05 ± 0.50b	2.25 ± 0.06c	14.48 ± 7.88
p-HPEA	7.89 ± 0.07ad	24.48 ± 3.89b	16.02 ± 1.79c	23.71 ± 7.44b	10.44 ± 0.01d	16.51 ± 7.53
vanillic acid	0.17 ± 0.01a	0.07 ± 0.01b	NQ	0.09 ± 0.03b	0.07 ± 0.01b	0.1 ± 0.05
vanillin	0.61 ± 0.05a	0.56 ± 0.14ad	0.47 ± 0.10ad	0.4 ± 0.10bd	0.85 ± 0.04c	0.58 ± 0.17
p-coumaric acid	0.5 ± 0.01a	0.8 ± 0.12b	0.36 ± 0.06c	0.42 ± 0.02ac	0.32 ± 0.02c	0.48 ± 0.19
ferulic acid	0.09 ± 0.00a	0.14 ± 0.01b	0.05 ± 0.01c	0.14 ± 0.00b	0.24 ± 0.01d	0.13 ± 0.07
luteolin	18.82 ± 0.06a	43.76 ± 1.97b	27.6 ± 0.88c	34.73 ± 1.12c	33.94 ± 1.04c	31.77 ± 9.25
apigenin	1.52 ± 0.01a	2.08 ± 0.30a	10.28 ± 1.30b	2.1 ± 0.71a	1.7 ± 0.34a	3.54 ± 3.78
hydroxytyrosol acetate	4.69 ± 0.14a	10.86 ± 9.23b	6.07 ± 0.94b	5.16 ± 0.50ab	5.61 ± 0.85ab	6.48 ± 2.5
3,4-DHPEA-EDA	9.38 ± 0.24ab	7.48 ± 0.32a	12.23 ± 2.07b	10.4 ± 1.12a	11.31 ± 1.86b	10.16 ± 1.83
pinoresinol	95.43 ± 1.89a	53.96 ± 2.38b	124.02 ± 20.56a	105.43 ± 10.70a	114.64 ± 18.10a	98.69 ± 27.17
dialdehydic form of oleuropein aglycon	26.97 ± 1.59a	11.1 ± 0.43b	34.84 ± 5.53a	29.62 ± 2.72a	32.2 ± 4.77a	26.95 ± 9.33
dialdehydic form of ligstroside aglycon	17.7 ± 0.25a	24.06 ± 0.73b	22.81 ± 3.55bc	19.39 ± 1.69ac	21.08 ± 3.02c	21.01 ± 2.55
total[a]	207.84 ± 23.46a	193.56 ± 14.96b	269.57 ± 30.53c	248.64 ± 0.00c	234.64 ± 28.46c	230.85 ± 30.62
TAP[a]	175.76 ± 9.19a	120.25 ± 23.07b	119.18 ± 4.86b	126.03 ± 6.18b	86.06 ± 5.51c	125.46 ± 32.21
α-tocopherol	128 ± 0.90a	145 ± 1.30b	139.53 ± 0.31b	139.21 ± 1.09b	138.1 ± 0.63b	137.97 ± 6.18

[a] Total, total amount by LC-MS quantified phenolic compounds; TAP, total amount of phenolic compounds determined by the Folin−Ciocalteu method and expressed in mg of gallic acid equiv/kg of olive oil (mg of GAE/kg); NQ, not quantified. Data represent average of triplicate measurements with indicated standard deviations. Different letters represent significant differences between samples ($p < 0.05$).

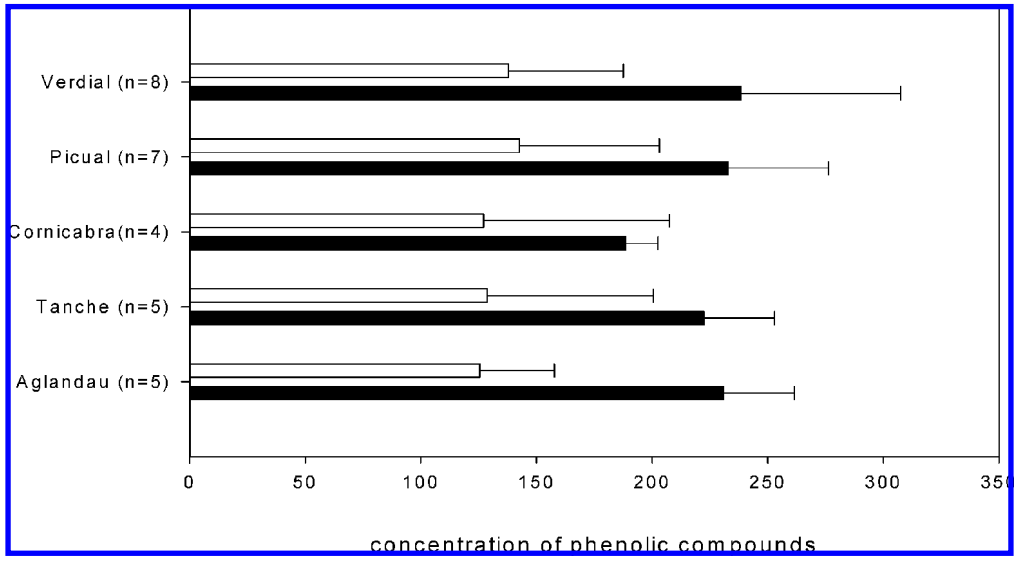

Figure 1. Average values of total quantified phenolic compounds (black bars, total, mg/kg) as well as their total amount as determined by the Folin−Ciocalteu method (white bars, TAP, mg of gallic acid equiv/kg) in each olive oil group (per cultivar).

levels of phenolic compounds and α-tocopherol in these oils and some well-characterized Spanish oils (Cornicabra, Picual, and Verdial de Huevar) has been made. A tentative predictive model for the total amount of phenolics was developed to further stress the importance of individual phenolic compounds and explain the relationships between individual phenolic compounds and their total amount as determined by a very common colorimetric method.

MATERIALS AND METHODS

Reagents and Standards. The following commercial products were used: gallic acid, *p*-coumaric acid, vanillic acid, and ferulic acid (Sigma-Aldrich NV/SA, Bornem, Belgium); *o*-coumaric acid, 3,4-dihydroxyphenylacetic acid, 4-hydroxyphenylacetic acid, and tyrosol (Acros Organics, Geel, Belgium); vanillin and apigenin (Fluka, Buchs, Switzerland). DL-α-Tocopherol and D-β-tocopherol (purity ≥ 95% by HPLC) were obtained from Calbiochem, Merck. Solid-phase cartridges (3 mL), packed with diol-bonded phase, were from Supelco, Discovery (Bellefonte, PA)−Sigma-Aldrich Family; Acrodisc syringe filters (Cr 13 mm; 0.45 μm PTFE Membrane), HPLC grade, were from Pall Life Science (Ann Arbor, MI). Hexane, methanol, ethyl acetate, and distilled water (all from Biosolve BV, Valkenswaard, The Netherlands), acetonitrile (Chem-Laboratory, Zedelgem, Belgium), and acetic acid (Fisher Chemicals, Leics, U.K.) were of LC-MS grade and used for LC-MS analysis. Folin−Ciocalteu reagent (Fluka) and sodium carbonate (Chem-Laboratory) were used for colorimetric analysis.

Oil Samples. EVOO samples ($n = 29$) from France (cultivars Aglandau, $n = 5$, and Tanche, $n = 5$) and Spain (cultivars Picual, $n = 7$, Verdial, $n = 8$, and Cornicabra, $n = 4$) were delivered within the European project Olive Track. Aglandau was produced in the region of Haute-Provence (grown at around 500 m altitude) and Tanche in Nyons (grown at 270 m). Cornicabra oil samples were from the Toledo region in Spain, whereas Picual and Verdial varieties were from the Jaen and Huelva regions (orchards were located from 65−585 m altitude).

Determination of the Content of Phenolic Compounds. Extraction of phenolic compounds was performed following the modified procedure described by Mateos et al. (*1*). The method is based on solid-phase extraction (SPE) with diol-phase cartridges using hexane and methanol for conditioning and hexane and hexane/ethyl acetate for washing the cartridge after the sample application. Finally, the extract was eluted with methanol, concentrated under vacuum, and reconstituted in methanol/water (1:1). Two internal standards were used, that is, *o*-coumaric and *p*-hydroxyphenylacetic acid. The first one was used as internal standard for the quantification of eight phenolic compounds (hydroxytyrosol, tyrosol, vanillic acid, vanillin, *p*-coumaric acid, ferulic acid, apigenin, and luteolin). For each compound the calibration curve

Table 2. Content of Phenolic Compounds (Milligrams per Kilogram) and α-Tocopherol (Milligrams per Kilogram) in Tanche Olive Oil

compound	FR t1	FR t2	FR t3	FR t4	FR t5	av
3,4-DHPEA	28.47 ± 0.51a	14.55 ± 9.86b	49.25 ± 4.38c	11.34 ± 0.20b	12.4 ± 0.51b	23.2 ± 16.11
p-HPEA	36.87 ± 0.58a	14.13 ± 4.96b	27.64 ± 1.66c	7.05 ± 0.16d	6.88 ± 0.07d	18.51 ± 13.29
vanillic acid	ND	0.08 ± 0.09a	0.13 ± 0.02b	NQ	NQ	0.11 ± 0.04
vanillin	0.23 ± 0.00ac	0.67 ± 0.19b	0.26 ± 0.02c	0.35 ± 0.05c	0.56 ± 0.03b	0.41 ± 0.19
p-coumaric acid	NQ	0.6 ± 0.05a	0.17 ± 0.02b	0.18 ± 0.01b	0.1 ± 0.00b	0.26 ± 0.23
ferulic acid	ND	0.13 ± 0.01	NQ	NQ	NQ	0.13 ± 0.01
luteolin	8.9 ± 0.21a	25.81 ± 5.50b	15.05 ± 0.42a	12.27 ± 0.11a	14.53 ± 0.69a	15.31 ± 6.35
apigenin	1.23 ± 0.85a	1.41 ± 0.11a	1.34 ± 0.03a	0.81 ± 0.13a	1.07 ± 0.14a	1.17 ± 0.24
hydroxytyrosol acetate	4.51 ± 0.55a	5.11 ± 0.52a	5.35 ± 0.19a	4.86 ± 0.64a	4.99 ± 0.50a	4.96 ± 0.31
3,4-DHPEA-EDA	9.09 ± 1.23a	10.3 ± 1.18a	10.77 ± 0.52a	9.79 ± 1.42a	10.05 ± 1.14a	10 ± 0.62
pinoresinol	92.19 ± 11.91a	104.41 ± 11.32a	109.25 ± 4.98a	99.23 ± 13.83a	101.92 ± 10.90a	101.4 ± 6.33
dialdehydic form of oleuropein	25.9 ± 3.09a	29.33 ± 2.90a	30.71 ± 1.22a	27.87 ± 3.61a	28.64 ± 2.78a	28.49 ± 1.79
aglycon	16.96 ± 1.95a	19.21 ± 1.81a	20.11 ± 0.76a	18.25 ± 2.28a	18.75 ± 1.74a	18.65 ± 1.17
total[a]	224.35 ± 24.71a	225.74 ± 26.00a	270.03 ± 28.49b	191.98 ± 24.61c	199.8 ± 25.20c	222.38 ± 30.49
TAP[a]	200.39 ± 2.91a	90.78 ± 8.89b	212.54 ± 0.68a	66.44 ± 3.46c	72.89 ± 7.59c	128.61 ± 71.76
α-tocopherol	238.89 ± 0.70a	213.92 ± 0.31b	229.85 ± 1.04a	201.95 ± 0.32b	225.15 ± 0.31a	221.95 ± 14.35

[a] Total, total amount by LC-MS quantified phenolic compounds; TAP, total amount of phenolic compounds determined by the Folin−Ciocalteu method and expressed in mg of gallic acid equiv/kg of olive oil (mg of GAE/kg); ND, not detected; NQ, not quantified. Data represent average of triplicate measurements with indicated standard deviations. Different letters represent significant differences between samples.

Table 3. Content of Phenolic Compounds (Milligrams per Kilogram) and α-Tocopherol (Milligrams per Kilogram) in Cornicabra Olive Oil

compound	SP c1	SP c2	SP c3	SP c4	av
3,4-DHPEA	25.93 ± 0.68a	30.82 ± 1.76ac	1.51 ± 0.07b	30.31 ± 3.04c	22.14 ± 13.93
p-HPEA	28.51 ± 1.41a	35.99 ± 2.12a	2.74 ± 0.08a	20.43 ± 2.33a	21.92 ± 14.28
vanillic acid	NQ	NQ	NQ	1.58 ± 0.02a	1.58 ± 0.02
vanillin	ND	ND	0.51 ± 0.02a	1.67 ± 0.18b	1.09 ± 0.82
p-coumaric acid	ND	ND	0.05 ± 0.01a	1.13 ± 0.05b	0.59 ± 0.76
ferulic acid	NQ	NQ	NQ	1.27 ± 0.03	1.27 ± 0.03
luteolin	11.57 ± 0.33a	11.23 ± 0.49a	12.02 ± 2.49a	5.16 ± 0.21b	10 ± 3.24
apigenin	5.68 ± 0.06a	4.97 ± 0.31a	3.05 ± 2.08a	2.55 ± 0.15a	4.06 ± 1.5
hydroxytyrosol acetate	5.14 ± 1.41a	5.14 ± 2.12a	5.08 ± 0.08a	1.77 ± 0.23b	4.28 ± 1.68
3,4-DHPEA-EDA	10.35 ± 1.37a	10.37 ± 3.44a	10.24 ± 1.47a	9.5 ± 4.26b	10.12 ± 0.41
pinoresinol	105 ± 14.09a	105.09 ± 34.18a	103.91 ± 15.03a	106.74 ± 29.30b	105.18 ± 1.17
dialdehydic form of oleuropein aglycon	29.52 ± 3.96a	29.49 ± 9.29a	29.21 ± 4.19a	6.49 ± 0.36b	23.68 ± 11.46
dialdehydic form of ligstroside aglycon	19.33 ± 2.60a	19.29 ± 5.99a	19.13 ± 2.74a	3.41 ± 0.15b	15.29 ± 7.92
total[a]	241.03 ± 29.69ac	252.39 ± 29.41a	187.45 ± 26.93b	192.01 ± 0.00c	218.22 ± 33.27
TAP[a]	173.02 ± 7.08a	191.78 ± 8.27a	206.2 ± 4.24b	182.77 ± 6.93c	188.44 ± 14.1
α-tocopherol	94.39 ± 2.98a	100.2 ± 8.65a	124.77 ± 0.18b	82.44 ± 2.30c	100.45 ± 17.82

[a] Total, total amount by LC-MS quantified phenolic compounds; TAP, total amount of phenolic compounds determined by the Folin−Ciocalteu method and expressed in mg of gallic acid equiv/kg of olive oil (mg of GAE/kg); ND, not detected; NQ, not quantified. Data represent average of triplicate measurements with indicated standard deviations. Different letters represent significant differences between samples ($p < 0.05$).

in the range of 0.01−0.1 mg/mL was obtained. The second standard based on the response factors (relationship between peak area and concentration) suggested by Mateos et al. (*16*) was used for quantification of the dialdehydic form of decarboxymethyl oleuropein aglycon, pinoresinol, 1-acetoxypinoresinol, and the aldehydic forms of oleuropein aglycon and ligstroside aglycon.

Separation and quantification of individual phenolic compounds were achieved by high-performance liquid chromatography (HPLC) with a diode array detector (DAD) at 280 nm. Further detection was done by mass spectrometry in an Agilent 1100 LC-MSD system (Agilent Technologies, Waldbronn, Germany). Using a Phenomenex C18 (ODS, Octadecyl) security guard and a Phenomenex Luna C18 (2) 100 Å column (4.6 mm i.d. × 250 mm; particle size = 10 μm) maintained at 35 °C, separation and elution of phenolic compounds were performed at a flow rate of 1.0 mL/min. A mobile phase was made of a mixture of 0.2% acetic acid in water (solvent A) with pH 3.1, methanol (solvent B), and acetonitrile (solvent C), where solvents B and C were mixed at 50:50, v/v. The solvent gradient was changed according to the following conditions: (1) 0−10 min, B/C = 2.5−15%; (2) 10−20 min, B/C = 15−17.5%; (3) 20−25 min, B/C = 17.5−20%; (4) 25−50 min, B/C = 20−35.5%; (5) 50−55 min, B/C = 35−50%; (6) 55−65 min, B/C = 50−50%; (7) 65−67 min, B/C = 50−2.5%; and (8) 67−72 min, B/C = 2.5−2.50%. Additionally, the detection was done by an Agilent G1946D (SL) quadrapole mass spectrometer (Agilent Technologies) equipped with an electrospray ionization (ESI) system and controlled by Agilent software v. A. 09.03. Nitrogen was used as nebulizing gas at a pressure of 50 psi, and the flow was adjusted to 13 L/min. The heated capillary and voltage were maintained at 350 °C and 4.1 kV, respectively. The full-scan mass spectra of the phenolic compounds were measured from *m*/*z* 100 to 1000. Mass spectrometry data were acquired in the negative ionization mode.

The Folin−Ciocalteu method (*17*) was used for assessing the amount of total phenols in olive oil (TAP). Briefly, dry phenolic extract eluted and dried after SPE was reconstituted in 1 mL of MeOH. This was added to a 100 mL flask previously filled with approximately 60 mL of distilled water, which was followed by the addition of 5 mL of diluted Folin−Ciocalteu reagent (1/10 dilution in distilled water). After 5 min, 15 mL of a 20% aqueous solution of Na_2CO_3 was added to increase the pH and distilled water was added to 100 mL. The reaction was allowed to develop during 2 h in dark conditions at room temperature. The absorbance of the complexes formed was read at 760 nm against methanol as a blank. The data are expressed as gallic acid equivalents per kilogram of olive oil (mg of GAE/kg) after generation of the calibration curve in the range of 0−400 mg/L of methanol.

Determination of Tocopherols. The α-tocopherol content was determined according to AOCS Official Method Ce 8-89 (*18*). Detection was performed with a UV detector set at 292 nm. For the quantification a calibration curve of α-tocopherol was made in the range of 0−60 mg/mL of hexane linearly relating the concentrations and their UV response (intercept and slope were 1.948 and 5.5654, respectively) with a regression coefficient of $R^2 = 0.998$.

Statistical Analysis. Correlation analysis of determined compounds

Table 4. Content of Phenolic Compounds (Milligrams per Kilogram) and α-Tocopherol (Milligrams per Kilogram) in Picual Olive Oil

compound	SP p1	SP p2	SP p3	SP p4	SP p5	SP p6	SP p7	av
3,4-DHPEA	19.83 ± 1.43a	21.2 ± 2.76a	21.99 ± 0.82a	37.81 ± 1.09a	4.17 ± 0.85b	3.62 ± 0.59b	28.75 ± 0.86c	19.62 ± 12.36
p-HPEA	10.35 ± 0.48ab	15.6 ± 1.73a	9.92 ± 0.35ab	15.43 ± 1.09ab	3.89 ± 0.71b	0.09 ± 0.03b	33.66 ± 0.43c	12.71 ± 10.84
vanillic acid	NQ	0.42 ± 0.07a	NQ	1.53 ± 0.01b	NQ	0.15 ± 0.03c	0.41 ± 0.01a	0.63 ± 0.61
vanillin	0.58 ± 0.14a	0.51 ± 0.04ac	0.56 ± 0.06a	0.67 ± 0.00bc	0.58 ± 0.13a	0.4 ± 0.04ac	0.33 ± 0.03ac	0.52 ± 0.12
p-coumaric acid	0.18 ± 0.14a	0.52 ± 0.06b	0.11 ± 0.00a	0.87 ± 0.02bc	0.16 ± 0.05a	0.25 ± 0.04a	0.79 ± 0.02c	0.41 ± 0.32
ferulic acid	ND	ND	ND	1.14 ± 0.00a	ND	ND	0.13 ± 0.00b	0.64 ± 0.71
luteolin	10.72 ± 1.94a	21.24 ± 2.70b	9.92 ± 0.45a	2.8 ± 0.35d	11.15 ± 2.47a	12.42 ± 0.63a	16.14 ± 0.70c	12.06 ± 5.69
apigenin	1.46 ± 0.39a	2.25 ± 0.34a	2.85 ± 0.13a	12.19 ± 0.88b	2.05 ± 0.97a	1.79 ± 0.36a	1.53 ± 0.08a	3.45 ± 3.88
hydroxytyrosol acetate	5.25 ± 0.48ac	8.02 ± 0.94b	6.36 ± 0.29a	9.27 ± 0.83b	4.65 ± 0.60c	5.01 ± 0.38ac	4.86 ± 0.39ac	6.2 ± 1.79
3,4-DHPEA-EDA	10.57 ± 1.53a	16.14 ± 1.82b	12.8 ± 0.43ab	2.05 ± 3.48c	9.37 ± 1.27a	10.08 ± 0.77a	9.8 ± 0.92a	10.12 ± 4.27
pinoresinol	107.31 ± 16.26ac	133.66 ± 17.88b	129.88 ± 4.61b.d	148.74 ± 7.99bc	95.03 ± 13.11ad	102.27 ± 8.33ad	99.35 ± 9.01ad	116.61 ± 20.64
dialdehydic form of oleuropein aglycon	30.19 ± 4.80ad	46 ± 4.93b	36.52 ± 1.55a	9 ± 0.50c	26.72 ± 3.70d	28.76 ± 2.51ad	27.92 ± 2.3 4ad	29.3 ± 11.2
dialdehydic form of ligstroside aglycon	19.78 ± 3.21ac	30.13 ± 3.21b	23.92 ± 1.10a	19.52 ± 1.10ac	17.5 ± 2.43c	18.84 ± 1.70ac	18.28 ± 1.48ac	21.14 ± 4.47
total[a]	216.3 ± 27.30a	295.78 ± 41.36b	254.84 ± 32.74a	261.02 ± 0.00ab	175.33 ± 24.29c	183.74 ± 26.16c	242.01 ± 25.14d	232.72 ± 43.43
TAP[a]	126.35 ± 0.48a	185.6 ± 1.73b	169.92 ± 0.35b	223.23 ± 9.19c	65.04 ± 9.02d	63.83 ± 4.99d	163.66 ± 0.43b	142.52 ± 60.59
α-tocopherol	169.32 ± 8.37ab	149.95 ± 0.52a	182.3 ± 2.97b	130.85 ± 1.03c	150.46 ± 7.01a	133 ± 12.40c	122.97 ± 1.66c	148.41 ± 21.54

[a] Total, total amount by LC-MS quantified phenolic compounds; TAP, total amount of phenolic compounds determined by the Folin−Ciocalteu method and expressed in mg of gallic acid equiv/kg of olive oil (mg of GAE/kg); ND, not detected; NQ, not quantified. Data represent average of triplicate measurements with indicated standard deviations. Different letters represent significant differences between samples ($p < 0.05$).

Table 5. Content of Phenolic Compounds (Milligrams per Kilogram) and α-Tocopherol (Milligrams per Kilogram) in Verdial Olive Oil

compound	SP v1	SP v2	SP v3	SP v4	SP v5	SP v6	SP v7	SP v8	av
3,4-DHPEA	27.19 ± 1.10af	29.38 ± 7.83af	15.61 ± 0.34b.d	19.45 ± 0.70fde	13.33 ± 3.50be	11.66 ± 0.74b.e	35.74 ± 1.31c	41.53 ± 1.39ac	24.24 ± 10.96
p-HPEA	44.54 ± 1.1	32.2 ± 9.69	12.26 ± 0.5	18.11 ± 0.29	19.25 ± 4.74	26.94 ± 0.35	37.22 ± 1.84	39.87 ± 1.04	28.8 ± 11.56
vanillic acid	0.01 ± 0.02	0.04 ± 0.02	NQ	0 ± 0.01	0.11 ± 0.07	0.22 ± 0.01	0.43 ± 0.03	0.46 ± 0.03	0.18 ± 0.2
vanillin	0.69 ± 0.01	0.43 ± 0.07	0.22 ± 0	0.23 ± 0	ND	ND	0.27 ± 0.01	0.27 ± 0	0.35 ± 0.18
p-coumaric acid	1.41 ± 0.01	0.57 ± 0.31	0.25 ± 0.02	0.21 ± 0.01	0.4 ± 0.14	NQ	0.75 ± 0.04	0.77 ± 0.03	0.62 ± 0.41
ferulic acid	1.48 ± 0.01	0.03 ± 0.04	ND	ND	ND	ND	0.19 ± 0.01	0.15 ± 0.01	0.46 ± 0.68
luteolin	2.26 ± 0.02a	16.57 ± 5.51b	13.03 ± 0.39ab	18.11 ± 0.41ab	18.51 ± 8.71b	21.55 ± 2.95b	20.2 ± 1.57b	19.09 ± 0.87b	16.17 ± 6.17
apigenin	3.07 ± 0.18ace	2.44 ± 0.36ace	1.74 ± 0.10ae	1.17 ± 0.09be	2.33 ± 1.13efg	2.59 ± 0.30ce	3.19 ± 0.27cdg	2.87 ± 0.19ef	2.43 ± 0.68
hydroxytyrosol acetate	5.62 ± 0.24a	5.61 ± 1.55a	4.51 ± 0.63b	ND	5.65 ± 1.62a	6.03 ± 0.55ac	6.22 ± 0.93c	6.78 ± 1.02c	5. 78 ± 0.7
3,4-DHPEA-EDA	2.05 ± 0.40a	11.33 ± 3.28b	9.09 ± 1.39b	9.26 ± 0.50b	11.36 ± 3.12b	12.14 ± 0.96b	12.55 ± 2.05b	13.67 ± 2.24b	10.18 ± 3.63
pinoresinol	16.86 ± 3.59a	114.87 ± 32.83b	92.12 ± 13.54b	93.96 ± 5.09b	115.17 ± 31.59b	123.17 ± 9.91b	127.21 ± 20.13b	13 8.57 ± 21.88b	102.74 ± 38.11
dialdehydic form of oleuropein aglycon	3.71 ± 0.59a	32.25 ± 8.96b	25.88 ± 3.55b	26.41 ± 1.37b	32.4 ± 8.97b	34.64 ± 2.97b	35.73 ± 5. 32b	38.92 ± 5.76b	28.74 ± 11.04
dialdehydic form of ligstroside aglycon	1.05 ± 0.26a	21.11 ± 5.79b	16.94 ± 2.25b	17.3 ± 0.89b	21.22 ± 5.90b	22.69 ± 2.01b	23.39 ± 3.38b	25.48 ± 3.66b	18.65 ± 7.68
total[a]	109.94 ± 0.00a	266.91 ± 30.09a	191.75 ± 23.33a	204.21 ± 26.41b	240.12 ± 31.43a	261.7 ± 34.07a	303.17 ± 32.10a	328. 51 ± 35.06a	238.29 ± 69.2
TAP[a]	140.29 ± 7.80a	112.2 ± 9.69ab	73.1 ± 21.21bc	123.1 ± 21.21c	123.35 ± 13.22c	119.24 ± 9.80cd	172.22 ± 1.84a	239.8 7 ± 1.04a	137.92 ± 49.6
α-tocopherol	158.56 ± 0.00a	174 ± 0.39b	199.54 ± 4.85c	141.61 ± 13.29a	161.03 ± 2.31	219.84 ± 4.65d	150.43 ± 2.11a	207.53 ± 1.96c	176.57 ± 28.87

[a] Total, total amount by LC-MS quantified phenolic compounds; TAP, total amount of phenolic compounds determined by the Folin−Ciocalteu method and expressed in mg of gallic acid equiv/kg of olive oil (mg of GAE/kg); ND, not detected; NQ, not quantified. Data represent average of triplicate measurements with indicated standard deviations. Different letters represent significant differences between samples ($p < 0.05$).

and calculated parameters, all of them being results of triplicate measurements, were performed using SPSS software package for Windows (SPSS 12.0). Pearson's correlation coefficients with a significance level of <0.05 were reported. To better explain the relationships between some variables, partial least-squares (PLS) regression analysis was done using The Unscrambler (CAMO Software AS), version 9.6. PLS is a method for relating the variations in one or several response variables to the variations of several predictors, with explanatory or predictive purposes. In this study, one response variable (TAP) was predicted by 15 predictors (13 phenolic compounds, their sum, and α-tocopherol). Data were preprocessed by using a full cross-validation technique where in the process of validation of the model one sample is kept out of the calibration and used for prediction. Also, root-mean-square error of prediction (RMSEP) as a measurement of an average difference between predicted and measured response values was calculated.

RESULTS AND DISCUSSION

Analytical Profile of Olive Oils. The contents of 13 phenolic compounds and of α-tocopherol were determined in the olive oil samples (**Tables 1–5**). As frequently reported, the main simple phenols found in all olive oil samples were hydroxytyrosol (htyr, 3,4-DHPEA) and tyrosol (tyr, *p*-HPEA). Other simple phenolics were vanillic acid (va), vanillin (van), *p*-coumaric acid (*p*-coum), and ferulic acid (fa), which were present in concentrations lower than 2 mg/kg in all samples. This is in accordance with previous reports (*19, 20*). However, whereas vanillin and *p*-coumaric acid were detected in almost all samples, ferulic acid was detected in Aglandau samples (and FR t2) and some Spanish samples (SP c4, SP p4, SP p7, SP v1, SP v2, SP v7, and SPv8).

Pinoresinol (pin), a lignan by structure, was detected in the highest amount in all olive oil samples. However, the amount of pinoresinol in olive oils of French origin was significantly lower than in those of Spanish origin ($p < 0.01$). No statistical difference was observed in pinoresinol content in Aglandau and Tanche olive oils (98.69 and 101.40 mg/kg, respectively). Likewise, Cornicabra, Picual, and Verdial oils had similar average amounts of this compound (119.23, 123.10, and 108.89 mg/kg, respectively). The pinoresinol content in the Picual variety was higher than that previously reported by García et al. (*21*), probably due to the differences in extraction and quantification procedures and/or the origin of the samples. The compound is generally presumed to be a plant defensive agent due to its antihelminthic and antifungal activities (*22*) and thus can be affected by environmental stress. In addition, pinoresinol also possesses phytoestrogenic activity, which may be important for human health (*23*).

Other identified and quantified compounds were hydroxytyrosol acetate (5.56 mg/kg average values in all samples; hac), the dialdehydic form of decarboxymethyl oleuropein aglycon (3,4-DHPEA-EDA), and the aldehydic form 3,4-DHPEA-EDA.

In contrast to the noticeable variation in the content of individual compounds, the variation in the total amount of phenolics (TAP) was significantly less pronounced (p value > 0.05, ANOVA). As illustrated in **Figure 1**, the average amounts of total phenolic content in Aglandau and Tanche varieties were similar. A slightly higher amount of TAP was observed in Cornicabra, whereas Picual and Verdial had the highest average amount of total phenolics as determined by Folin−Ciocalteu (>230 mgof GAE/kg).

In regard to α-tocopherol (αT) content, olive oil from the Tanche variety had the highest average amount (221 ± 13 mg/kg, p value < 0.01, ANOVA), followed by Picual (195 ± 20 mg/kg), Verdial (178 ± 27 mg/kg), and Aglandau (138 ± 6 mg/kg), whereas Cornicabra olive variety had the lowest amount of α-tocopherol (105 ± 20 mg/kg). Accordingly, Salvador et al. (*13*) suggested that this oil variety may have lower tocopherol content than other Spanish varieties. Results presented here suggest that the content of α-tocopherol in Cornicabra oils is lower than in the French Aglandau and Tanche varieties. The evaluated phenolic profiles (and αT) may contribute to further extend current databases on phenolic compounds in European olive oils.

Correlation of Phenolic Compounds. Among individual phenolic compounds, hydroxytyrosol and tyrosol correlated significantly ($r = 0.648$, $p < 0.01$). A similar correlation was observed between vanillic acid and vanillin ($r = 0.580$, $p < 0.01$), vanillic acid and *p*-coumaric acid ($r = 0.548$, $p < 0.01$), vanillic acid and ferulic acid ($r = 0.643$, $p < 0.01$), vanillin and ferulic acid ($r = 0.640$, $p < 0.01$), and finally *p*-coumaric and ferulic acid ($r = 0.808$, $p < 0.01$). Some of these phenolic acids, in particular vanillic acid and ferulic acid, correlated moderately to hydroxytyrosol acetate ($r = 0.642$, $p < 0.01$, and $r = 0.613$, $p < 0.05$, respectively).

Correlations were also found for the more complex phenolic compounds determined in olive oil. Levels of pinoresinol were higher in oils with higher amounts of the aldehydic form of ligstroside aglycon ($r = 0.754$, $p < 0.01$). Pinoresinol was not significantly correlated to the major simple phenolic compound, hydroxytyrosol. These compounds not only have different biosynthetic pathways, but they behave differently during storage. The contents of htyr and tyr may increase during storage, whereas pinoresinol content is not affected (*24*). Therefore, lack of correlation between these compounds is not surprising. On the other hand, in all olive oil samples, the highly correlated aldehydic forms of ligstroside aglycone and oleuropein aglycone ($r = 0.912$, $p < 0.01$) were also highly correlated to the dialdehydic form of decarboxymethyl oleuropein aglycon ($r > 0.90$, $p < 0.01$, for both aldehydic forms). In contrast, these compounds were not in correlation with hydroxytyrosol or tyrosol. Although it has been suggested that during storage the hydrolysis of secoiridoid derivatives, such as those mentioned above, would cause an increase in the level of hydroxytyrosol and tyrosol (*25*), bringing them in a negative correlation, this relationship was not found in this study. Different analytical standards (*o*-coumaric acid for htyr and tyr vs hydroxyphenyl acetic acid for the others) used for calculating the compound quantities may explain these discrepancies (*5*). The detector responses for these compounds show differences preventing the quantification of the real concentration (*26*).

Within the individual phenolic compounds, hydroxytyrosol correlated best to the total amount of phenolic compounds as determined by the Folin−Ciocalteu method (TAP) ($r = 0.829$, $p < 0.01$), whereas pinoresinol correlated best to the sum of phenolic compounds determined by HPLC ($r = 0.815$, $p < 0.001$). Pinoresinol as well as tyrosol and vanillic acid correlated moderately to TAP ($r = 0.436$, $p = 0.018$, $r = 0.514$, $p < 0.001$, and $r = 0.440$, $p = 0.017$, respectively), whereas the dialdehydic forms of decarboxymethyl oleuropein aglycone, ligstroside aglyconin, and oleuropein aglycone correlated well to the sum of quantified phenolics ($r = 0.636$, $r = 0.673$, and $r = 0.737$; $p < 0.01$ for all three). The rest of the components did not show any statistically significant correlation to the total amount of phenolic compounds in the oil (either total or TAP). The highest contribution to the TAP can as such be ascribed to tyrosol and hydroxytyrosol, which can be explained by the structure-related reaction mechanism of the Folin−Ciocalteu reagent with phenolic compounds (*17*). It is noteworthy to

mention that only a moderate correlation was found between the results of the total phenolic content determined by HPLC and by Folin−Ciocalteu ($r = 0.64$ and $p < 0.01$).

Neither any of the phenolic compounds nor their sum (both LC-MS and TAP) was correlated with the amount of α-tocopherol. Whereas the content of α-tocopherol in olive oil is highly variety dependent (*27*), clear evidence regarding the dependence of phenolic compounds on the olive variety is still not established (*28, 29*). Despite this, there are some indications that phenolic compounds may be considered as markers of merely the geographic origin, in particular the location of the orchards (*30*).

With regard to the impact of geographic origin on phenolic composition of olive oil, the correlation between altitude and the phenolic compounds was assessed. For this purpose, only French olive oil samples were taken into account because they have not been commonly discussed in the literature and may offer new insights on this item. The correlation between the total amount of phenolic compounds quantified in French samples and the altitude (Aglandau was grown around 500 m and Tanche at 270 m) at which olive orchards were located was moderate or completely lacking. Of all individual phenolics, luteolin was found to be highly correlated to this parameter ($r = 0.76$, $p < 0.01$), whereas the correlation of α-tocopherol to altitude was negative and significant ($r = -0.97$ and $p < 0.04$). This in turn may indicate that olive oil produced from olives cultivated at lower altitudes may have higher amounts of α-tocopherol, in contrast to those from the higher regions, which can contain higher amounts of luteolin. The effect of altitude on olive oil quality has been discussed by some authors for fatty acids and sterols (*16*) but has not yet been fully reported for phenolics (*7*). Nevertheless, for better evaluation of the influence of this factor on the content of phenolic compounds in olive oil a more extensive study would be necessary.

Regression Analysis. To explain better the relationship between total amount of phenolics (TAP) and individual phenolic compound, PLS regression analysis was performed. PLS regression specifies the (linear) relationship between a dependent variable and a set of predictor variables generating a set of orthogonal basis vectors, or "loadings", suggesting the number of principal components to be used for evaluation and providing a useful platform for model interpretation and outlier detection. Using this statistical technique, coefficients for predictions of TAP were computed and are presented in eq 1, whereby hydroxytyrosol (*htyr) and total amount of quantified phenolics (*total) were significant variables.

$$\mathrm{TAP} = 0.41 + 0.78{*}\mathrm{htyr} + 0.16\mathrm{tyr} + 0.16\mathrm{va} + 0.11\mathrm{van} - 0.45\mathrm{p\text{-}coum} + 0.09\mathrm{fa} - 0.09\mathrm{lut} + 0.07\mathrm{apig} + 0.14\mathrm{hac} + 0.02\ \mathrm{ddoa} - 0.04\mathrm{pin} - 0.14\mathrm{aoa} + 0.23\mathrm{ala} + 0.19{*}\mathrm{total} - 0.27\alpha\mathrm{T} \quad (1)$$

PLS regression for TAP as dependent (respond) variable confirmed the significant importance of hydroxytyrosol followed by the total amount of quantified phenolics. Most of the other compounds had a negligible effect except for *p*-coumaric acid and α-tocopherol, which were inversely related to TAP. The correlation between measured and predicted values was high ($r = 0.94$), with an estimated prediction error of 17.6 (RMSEP). The coefficients for estimation of TAP are based on a calculated principal component analysis (PCA), using a model of six principal components. These results are in accordance with the correlation coefficients reported above. In addition, the regression coefficients calculated by PLS facilitate the prediction of the TAP, taking into account the correlations of all independent variables used, in particular phenolic compounds, α-tocopherol, and total. This is also important when the correlation between Tap and total is evaluated because different reports about their relationship may be found either high (*31*) or moderate as in this study. This prediction can be used in the quality evaluation of commercial samples where sometimes the total amount of phenolics (TAP) is reported on the label. Furthermore, the same principle can be applied to predict other quality parameters, such as oxidative stability, which could further be addressed in future studies.

ACKNOWLEDGMENT

We epecially thank Dr. Pilar Hernandez for providing all technical details about Spanish olive oil samples.

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Received for review August 14, 2007. Revised manuscript received April 21, 2008. Accepted April 29, 2008. We thank the Special Research Fund of University of Ghent for financially supporting this research under Project BW/04749/01 and in relation to Bilateral Research Fund Flanders−Romania BOF-2003-2005, B/04238/01. We express gratitude for the use of olive oil samples that were collected within the scope of the project financially supported by the Commission of the European Communities, specific RTD program "Quality of Life and Management of Living Resources", QLK-CT-2002-02386, Traceability of origin and authenticity of olive oil by combined genomic and metabolomic approaches (OLIV-TRACK).

JF0724419