Evaluation of Virgin Olive Oil Bitterness by Quantification of Secoiridoid Derivatives

Raquel Mateos, Arturo Cert, M. Carmen Pérez-Camino, and José M. García*

Instituto de la Grasa (Consejo Superior de Investigaciónes Científicas), E-41012 Sevilla, Spain

ABSTRACT: The bitterness of the main compounds identified in the phenolic extract of virgin olive (*Olea europaea* L.) oils has been sensory-tested. The aldehydic form of oleuropein aglycone (AOA) was responsible for this attribute. Correlations between the sensory bitterness and concentrations of secoiridoid derivatives, analyzed separately or in different combinations, were obtained for olive oils from different olive varieties. The best correlation obtained corresponds to AOA content ($r = 0.96$; $P = 1.83 \times 10^{-17}$) in the concentration range of 0.03 to 0.5 mmol/kg. AOA concentrations ≥0.5 mmol/kg produce sensory saturation of this attribute. The correlation with AOA concentration was better than that with the absorbance of the phenolic extract at 225 nm. Therefore, the equation obtained allows the evaluation of the bitterness in virgin olive oils by HPLC analysis of the phenolic extract using detection at 280 nm.

Paper no. J10590 in *JAOCS 81,* 71–75 (January 2004).

KEY WORDS: Bitter taste, hydroxytyrosol, phenolic compounds, secoiridoid derivatives, sensory evaluation, virgin olive oil.

Olive oil is called "virgin" if it is obtained from fruits crushed by press or centrifugation. It is an oily fruit juice, whose widely recognized nutritional value is related to its protective action against cardiovascular diseases and cancer (1–3). The consumption of this product is increasing in countries such as the United States, Canada, and Japan and in the European Community. However, consumers in these countries are not habituated to some of the sensory attributes of this oil, such as bitterness, astringency, or pungency. Virgin olive oils (VOO) with a high intensity of these attributes are hardly marketable in these countries. Consequently, these oils must be blended with nonbitter VOO or with refined olive oil, constituting a "coupage" or an "olive oil," respectively. The standard method of analyzing the bitter taste of olive oil is by sensory analysis using a panel of tasters (4). However, an analytical panel is not likely to be available, since a permanent staff of trained tasters and a highly specialized panel chief is necessary. For this reason, methods for the evaluation of the bitterness level based on physicochemical determinations would be very useful for the industry.

Several authors have found a strong relationship between these sensory attributes and the content of phenolic compounds of the olive oils (5–8). Gutiérrez *et al.* (9) proposed the use of absorbance at 225 nm of the phenolic extract obtained from VOO for evaluation of bitter taste, since a good relationship with bitterness evaluated by an analytical panel was found. However, several nonbitter phenolic compounds absorb at 225 nm; consequently, this estimation is not appropriate for comparing bitterness of oil samples obtained from olive varieties with very different profiles in phenolic compounds, such as Picual and Arbequina.

The main objective of this work was to the identify the phenolic compounds actually responsible for olive oil bitterness and, with this information, elaborate a new method of evaluating the level of intensity of this important sensory attribute.

*To whom correspondence should be addressed at Instituto de la Grasa (CSIC), Avda. Padre García Tejero, 4, 41012 Sevilla. Spain.
E-mail: jmgarcia@cica.es

EXPERIMENTAL PROCEDURES

VOO samples. VOO obtained from olive (*Olea europaea* L., cvs. Arbequina, Cobrançosa, Hojiblanca, Manzanilla, Picual, and Verdial) varieties growing in Portugal, Tunisia, and Spain were supplied by industrial oil mills located in the aforementioned countries.

Reference compounds. The following commercial products were used: *p*-Hydroxyphenylacetic, *o*-, *p*-coumaric, and vanillic acids; vanillin; and luteolin were obtained from Sigma Chemical Co. (St. Louis, MO); apigenin was from Fluka AG (Buchs, Switzerland); ferulic and cinnamic acids were from Aldrich (Steinheim, Germany); and 2-(4′-hydroxyphenyl)ethanol (tyrosol) was obtained from Janssen Chemical Co. (Beerse, Belgium).

The following compounds were isolated or synthesized: 2-(3′,4′-Dihydroxyphenyl)ethanol (hydroxytyrosol) was synthesized from 3,4-dihydroxyphenylacetic acid by reduction with $LiAlH_4$ (10); 2-(3′,4′-dihydroxyphenyl)ethyl acetate (hydroxytyrosyl acetate) and 2-(4′-hydroxyphenyl)ethyl acetate (tyrosyl acetate) were obtained from hydroxytyrosol and tyrosol, respectively, by enzymatic transesterification with ethyl acetate (11); the secoiridoid derivative, an aldehydic form of oleuropein aglycone (AOA), was obtained by enzymatic hydrolysis of oleuropein with β-glucosidase from almonds (Sigma Chemical Co.) (12). This compound was purified by fractionation on a silica gel column using dichloromethane/acetone/hexane (3:2:5, by vol) as mobile phase. NMR data were in accordance with those reported by Montedoro *et al.* (13). Elenolic acid was obtained from oleuropein by hydrolysis with 1 N sulfuric acid at 55°C (14).

Analytical materials and reagents. All solvents and reagents were of analytical grade unless otherwise stated. Acetonitrile (far-UV), acetic acid, and methanol were of HPLC

grade (Romil Ltd., Cambridge, United Kingdom). Solid-phase extraction (SPE) cartridges (3 mL), packed with diol-bonded phase, were from Supelco (Bellefonte, PA). Ethyl alcohol was from Merck, KGaA (Darmstadt, Germany).

Bitterness test. The bitter taste of phenolic compounds was evaluated by the bitterness test described by Walter *et al.* (14). The bitterness of each compound of the phenolic fraction was evaluated by eight trained tasters. A Whatman filter paper was cleaned with ethyl alcohol and cut into squares of sides of 1 cm. Then 0.1 mL of the compound dissolved in ethanol (0.05 mmol/L) was placed on the paper and alcohol was evaporated. Squares of paper in which the compound was absorbed were placed by the panelists on their tongues to perceive the presence or absence of this attribute. Blank squares of paper were given to tasters first, and then a series of five papers containing increasing levels of the compound, using concentrations of 0.05, 0.10, 0.15, 0.20, and 0.25 mmol/L. Panelists were asked to discontinue the test at the first level where bitterness was detected.

Sensory evaluation of bitterness. All analyses of bitterness of VOO samples collected in Table 1 were determined by the same group of trained tasters according to European Commission Regulation EEC/2568/91 (4), using a structural scale of 6 points, where 0 means the absence of the attribute, 1 simple perception, 2 light presence, 3 middle presence, 4 strong intensity, and 5 the highest intensity.

Determination of phenolic compounds. The phenolic fraction was isolated by SPE and analyzed by RP-HPLC using a diode array UV detector (15).

Quantification of phenols, and the secoiridoid derivatives in particular, was carried out at 280 nm, and the results are expressed in millimol/kg.

Determination of the intensity of bitterness (IB). IB was determined by the method proposed by Gutiérrez *et al.* (9). A sample of 1.00 ± 0.01 g VOO was dissolved in 4 mL of hexane and passed through the C_{18} column previously activated with methanol (6 mL) and washed with hexane (6 mL). After elution, 10 mL of hexane was added to eliminate the fat, and then the retained compounds were eluted with methanol/water (1:1), collecting this fraction in a tared 25-mL beaker. The absorbance of the extract was measured at 225 nm against methanol/water (1:1) in a 1 cm cell. The results are expressed as the absorbance of 1 g in 100 g (K_{225}). IB was calculated by the following expression: IB = 13.33 K_{225} – 0.837.

Statistical analysis. Correlation parameters and equations were automatically calculated using CoStat 5.0 (CoHort Software, Monterey, CA).

RESULTS AND DISCUSSION

Simple components of the phenolic fraction of olive oil, such as hydroxytyrosol, tyrosol, vanillic acid, vanillin, *p*-coumaric acid, ferulic acid, cinnamic acid, elenolic acid, hydroxytyrosyl acetate, tyrosyl acetate, luteolin, and apigenin were evaluated by using the content usually found in olive oil for these compounds (approximately 0.25 mmol/L) as the highest concentration, following the bitterness test (14). None of them showed a bitter taste. On the other hand, the secoiridoid derivative AOA showed a high intensity of this attribute, since all tasters detected it at the initial concentration (0.05 mmol/L). The aldehydic form of ligstroside aglycone (ALA), the dialdehydic forms of decarboxymethyl oleuropein aglycone (DOA), and ligstroside aglycone (DLA) were not isolated from olive oil in large enough amounts for quantification. Finally, Arbequina VOO (sample no. 6), containing significant amounts of pinoresinol and 1-acetoxypinoresinol (0.15 mmol/kg) and flavones (0.05 mmol/kg) and a very low concentration of secoiridoid derivatives (0.04 mmol/kg), did not have a bitter taste.

The bitterness of VOO from different varieties evaluated by the panel of tasters (4) and the secoiridoid derivative composition determined by HPLC (15) are presented in Table 1. A relationship between the IB of oils and the influence of each one of the secoiridoid derivatives of hydroxytyrosol and tyrosol was established. In our opinion, the most valuable information can be obtained from oil samples with a lower IB. Thus, oil sample number 2, with a low IB (1.0), had a relatively high DOA content (0.44 mmol/kg). In the same way, oil sample number 5, with an even lower level of IB (0.5), had a relatively high DLA content (0.42 mmol/kg). The contribution of DOA and DLA molecules to the bitter taste of the olive oil appears to be very slight. On the other hand, oil sample numbers 7, 8, 9, 10, 14, 25, and 32, showing an extreme IB (5 on a 5-point scale), were not useful to study a possible linear regression between IB and the content of secoiridoid derivatives, since this limit value (5.0) is the same for different saturating concentrations of these compounds; consequently, they should not be included in this study. Correlation between IB and the concentrations of these compounds was studied, taking into account each one individually and in all possible combinations (Table 2). The best correlation obtained corresponded to the AOA concentration considered alone. The other secoiridoid derivatives also were significantly correlated with IB, but less strongly than AOA, because all of them were significantly correlated with AOA. ALA did not significantly correlate with bitter taste. For instance, oil sample 36 was evaluated as 3.3 in the scale of IB, and contained 0.51 mmol/kg of ALA and 0.33 mmol/kg of AOA, whereas oil sample 32 was extremely bitter and contained 0.34 mmol/kg of ALA and 0.47 mmol/kg of AOA. A similar pattern was noted in comparing samples 15 and 16 or samples 28 and 29. This pattern seems to indicate that ALA is secondary compared to the contribution of AOA to bitter taste. Oils with concentrations of AOA over 0.5 mmol/kg gave the maximum bitterness value (or 5), indicating that the saturation level was reached (Fig. 1). A linear relationship ($r = 0.96$, $P = 1.83 \times 10^{-17}$) occurred between sensory bitterness and AOA concentration in the 0.0 to 0.5 mmol/kg range. However, the high gradient of the linear function between 0 and 0.5 on the bitterness scale indicated that small amounts of these compounds can be easily perceived by tasters assigning a value of 0 or 0.5. For this reason, these results allow evaluation of bitterness by means of one analytical determination of AOA concentration, where

TABLE 1
Values of Bitterness and Concentration of Each Secoiridoid Derivative Expressed in mmol/kg of Different Extra Virgin Olive Oils[a]

Sample number	Variety	Bitterness (0–5)[b]	DOA[c] (mmol/kg)	DLA[d] (mmol/kg)	AOA[e] (mmol/kg)	ALA[f] (mmol/kg)	Total secoiridoid derivatives (mmol/kg)	IB[g]
1	Arbequina	2.0 ± 0.3	0.09 ± 0.01	0.06 ± 0.02	0.15 ± 0.02	0.16 ± 0.03	0.46 ± 0.05	0.80 ± 0.05
2	Arbequina	1.0 ± 0.3	0.44 ± 0.08	0.16 ± 0.04	0.04 ± 0.01	0.03 ± 0.00	0.67 ± 0.08	1.38 ± 0.08
3	Arbequina	0.7 ± 0.3	0.32 ± 0.05	0.37 ± 0.04	0.04 ± 0.00	0.08 ± 0.02	0.81 ± 0.06	1.70 ± 0.09
4	Arbequina	0.6 ± 0.1	0.15 ± 0.04	0.14 ± 0.01	0.04 ± 0.00	0.02 ± 0.01	0.35 ± 0.04	0.51 ± 0.06
5	Arbequina	0.5 ± 0.1	0.13 ± 0.04	0.42 ± 0.02	0.03 ± 0.00	0.05 ± 0.00	0.63 ± 0.07	1.79 ± 0.07
6	Arbequina	0.0 ± 0.0	0.01 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.01	0.10 ± 0.03
7	Cobrançosa	5.0 ± 0.0	2.10 ± 0.05	0.42 ± 0.06	2.92 ± 0.05	0.58 ± 0.03	6.02 ± 0.02	15.60 ± 0.09
8	Cobrançosa	5.0 ± 0.0	1.23 ± 0.08	0.74 ± 0.09	2.17 ± 0.06	0.10 ± 0.01	4.24 ± 0.06	5.80 ± 0.04
9	Hojiblanca	5.0 ± 0.0	2.87 ± 0.09	0.51 ± 0.05	0.70 ± 0.09	0.11 ± 0.01	4.19 ± 0.09	15.60 ± 0.08
10	Hojiblanca	5.0 ± 0.0	1.84 ± 0.05	0.33 ± 0.04	0.46 ± 0.01	0.06 ± 0.02	2.69 ± 0.09	6.50 ± 0.09
11	Hojiblanca	2.5 ± 0.3	0.58 ± 0.06	0.96 ± 0.04	0.28 ± 0.04	0.32 ± 0.01	2.14 ± 0.09	5.20 ± 0.05
12	Hojiblanca	2.5 ± 0.2	0.48 ± 0.03	0.37 ± 0.05	0.21 ± 0.04	0.19 ± 0.02	1.25 ± 0.08	0.46 ± 0.04
13	Hojiblanca	0.9 ± 0.1	0.28 ± 0.05	0.17 ± 0.06	0.09 ± 0.01	0.04 ± 0.01	0.58 ± 0.06	0.88 ± 0.04
14	Manzanilla	5.0 ± 0.0	1.23 ± 0.04	1.35 ± 0.08	1.20 ± 0.04	1.06 ± 0.03	4.84 ± 0.09	12.10 ± 0.04
15	Manzanilla	4.5 ± 0.4	0.43 ± 0.03	0.35 ± 0.04	0.47 ± 0.05	0.24 ± 0.05	1.49 ± 0.04	5.30 ± 0.05
16	Manzanilla	4.5 ± 0.6	0.40 ± 0.05	0.30 ± 0.02	0.46 ± 0.05	0.37 ± 0.04	1.53 ± 0.04	5.00 ± 0.06
17	Manzanilla	4.0 ± 0.5	0.40 ± 0.05	0.36 ± 0.05	0.43 ± 0.08	0.22 ± 0.01	1.41 ± 0.08	5.00 ± 0.06
18	Manzanilla	4.0 ± 0.5	0.44 ± 0.04	0.35 ± 0.06	0.43 ± 0.06	0.22 ± 0.03	1.44 ± 0.08	5.00 ± 0.06
19	Manzanilla	3.5 ± 0.5	0.12 ± 0.03	0.19 ± 0.02	0.40 ± 0.04	0.22 ± 0.05	0.93 ± 0.07	4.75 ± 0.09
20	Manzanilla	3.0 ± 0.3	0.36 ± 0.08	0.31 ± 0.05	0.40 ± 0.03	0.21 ± 0.04	1.28 ± 0.07	4.88 ± 0.09
21	Manzanilla	3.0 ± 0.4	0.18 ± 0.01	0.20 ± 0.05	0.39 ± 0.08	0.12 ± 0.03	0.89 ± 0.05	3.75 ± 0.07
22	Manzanilla	2.7 ± 0.4	0.31 ± 0.04	0.24 ± 0.04	0.35 ± 0.04	0.21 ± 0.06	1.11 ± 0.04	4.32 ± 0.04
23	Manzanilla	2.5 ± 0.5	0.22 ± 0.08	0.15 ± 0.04	0.22 ± 0.03	0.13 ± 0.03	0.72 ± 0.06	3.25 ± 0.04
24	Manzanilla	2.2 ± 0.6	0.20 ± 0.05	0.10 ± 0.04	0.19 ± 0.02	0.18 ± 0.04	0.67 ± 0.06	2.50 ± 0.08
25	Picual	5.0 ± 0.3	0.57 ± 0.04	0.44 ± 0.04	0.50 ± 0.05	0.40 ± 0.01	1.91 ± 0.05	3.75 ± 0.04
26	Picual	4.2 ± 0.5	0.47 ± 0.04	0.45 ± 0.04	0.44 ± 0.03	0.16 ± 0.02	1.52 ± 0.05	3.80 ± 0.04
27	Picual	3.0 ± 0.4	0.11 ± 0.04	0.20 ± 0.05	0.40 ± 0.04	0.10 ± 0.01	0.81 ± 0.09	3.50 ± 0.06
28	Picual	2.5 ± 0.4	0.28 ± 0.05	0.52 ± 0.06	0.21 ± 0.05	0.26 ± 0.02	1.27 ± 0.08	2.90 ± 0.04
29	Picual	2.0 ± 0.4	0.36 ± 0.08	0.22 ± 0.04	0.20 ± 0.04	0.06 ± 0.03	0.84 ± 0.09	0.84 ± 0.02
30	Picual	2.0 ± 0.4	0.16 ± 0.07	0.04 ± 0.01	0.22 ± 0.03	0.05 ± 0.01	0.47 ± 0.06	2.37 ± 0.06
31	Picual	0.5 ± 0.1	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.05 ± 0.01	1.37 ± 0.05
32	Verdial	5.0 ± 0.0	0.34 ± 0.06	0.75 ± 0.03	0.47 ± 0.08	0.34 ± 0.03	1.90 ± 0.09	9.10 ± 0.08
33	Verdial	3.5 ± 0.4	0.40 ± 0.05	0.17 ± 0.03	0.42 ± 0.04	0.15 ± 0.02	1.14 ± 0.07	6.80 ± 0.07
34	Verdial	3.5 ± 0.6	0.40 ± 0.04	0.26 ± 0.03	0.45 ± 0.04	0.13 ± 0.05	1.24 ± 0.08	6.55 ± 0.07
35	Verdial	3.5 ± 0.4	0.30 ± 0.01	0.36 ± 0.07	0.35 ± 0.02	0.23 ± 0.03	1.24 ± 0.06	7.00 ± 0.07
36	Verdial	3.3 ± 0.4	0.14 ± 0.01	0.49 ± 0.05	0.33 ± 0.05	0.51 ± 0.04	1.47 ± 0.05	7.52 ± 0.05
37	Verdial	3.3 ± 0.3	0.31 ± 0.03	0.17 ± 0.06	0.42 ± 0.06	0.12 ± 0.04	1.02 ± 0.04	6.25 ± 0.04
38	Verdial	2.8 ± 0.5	0.09 ± 0.01	0.06 ± 0.01	0.26 ± 0.07	0.05 ± 0.01	0.46 ± 0.05	5.85 ± 0.03
39	Verdial	2.7 ± 0.5	0.11 ± 0.02	0.36 ± 0.05	0.22 ± 0.08	0.30 ± 0.06	0.99 ± 0.05	5.00 ± 0.09
40	Verdial	2.5 ± 0.2	0.09 ± 0.02	0.08 ± 0.01	0.20 ± 0.04	0.09 ± 0.01	0.46 ± 0.02	6.00 ± 0.09
41	Verdial	2.3 ± 0.4	0.09 ± 0.02	0.19 ± 0.05	0.18 ± 0.02	0.14 ± 0.03	0.60 ± 0.06	5.50 ± 0.04
42	Verdial	1.9 ± 0.4	0.06 ± 0.03	0.26 ± 0.05	0.12 ± 0.04	0.12 ± 0.03	0.56 ± 0.02	4.12 ± 0.05
43	Verdial	1.4 ± 0.3	0.01 ± 0.01	0.04 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.19 ± 0.06	4.12 ± 0.06
44	Verdial	1.2 ± 0.6	0.01 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.01	0.15 ± 0.04	4.00 ± 0.09
45	Verdial	1.1 ± 0.5	0.02 ± 0.00	0.04 ± 0.00	0.06 ± 0.00	0.07 ± 0.02	0.19 ± 0.04	3.25 ± 0.08
46	Verdial	1.0 ± 0.2	0.01 ± 0.00	0.02 ± 0.00	0.05 ± 0.00	0.06 ± 0.02	0.14 ± 0.04	3.25 ± 0.07

[a]Each point is the mean value ± SD of three replicates.
[b]Scale from 0, absence of the attribute, to 5, the highest intensity.
[c]Dialdehydic form of decarboxymethyl oleuropein aglycone.
[d]Dialdehydic form of decarboxymethyl ligstroside aglycone.
[e]Aldehydic form of oleuropein aglycone.
[f]Aldehydic form of ligstroside aglycone.
[g]IB (intensity of bitterness) = 13.33 K_{225} − 0.837.

for values higher than 0.5 mmol/kg, bitterness is 5; for values included in the range between 0.03 and 0.5 mmol/kg, the level of bitterness is described by the following equation:

$$\text{bitterness} = 0.51 + 7.99\ \text{AOA} \qquad [1]$$

where AOA is expressed as mmol/kg.

This measurement of bitterness can be useful for preparing coupages or olive oil mixtures of very bitter VOO with nonbitter VOO or refined olive oils, respectively.

Equation 1 shows a very good correlation with the sensory bitterness data for each variety considered separately and also for all varieties together. Furthermore, IB obtained by absorbance at 225 nm gave poorer correlations with sensory

TABLE 2
Correlation Between Sensory-Evaluated Bitterness of 39 Different Virgin Olive Oils and Their Respective Contents of Different Combinations of Secoiridoid Derivatives

Variable	r^a	$P\ (r = 0)^b$
DOA	0.58	1.15×10^{-4}
DLA	0.39	1.4×10^{-2}
AOA	0.96	1.83×10^{-17}
ALA	0.68	2.22×10^{-6}
DOA + DLA	0.55	2.92×10^{-4}
DOA + AOA	0.85	6.25×10^{-12}
DOA + ALA	0.75	4.87×10^{-8}
DLA + AOA	0.78	4.38×10^{-9}
DLA + ALA	0.54	3.97×10^{-4}
DOA + DLA + AOA	0.77	1.12×10^{-8}
DOA + AOA + ALA	0.89	1.86×10^{-14}
DOA + DLA + ALA	0.64	1.35×10^{-5}
DLA + AOA + ALA	0.79	2.05×10^{-9}
DOA + DLA + AOA + ALA	0.80	1.44×10^{-9}

[a]Linear regression coefficient.
[b]Probability that $r = 0$. For abbreviations see Table 1.

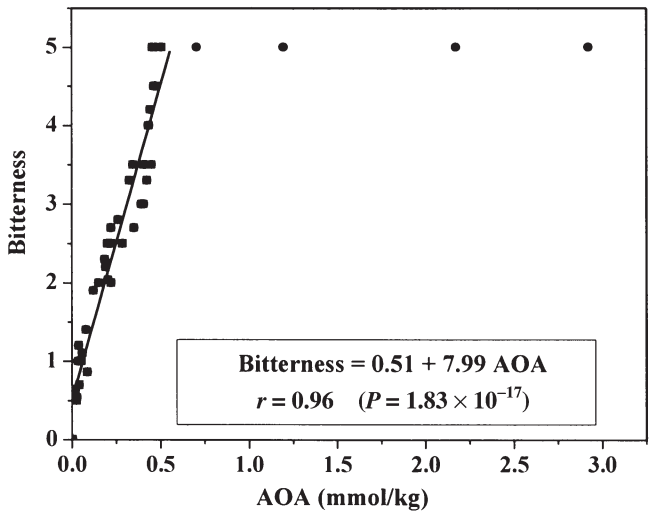

FIG. 1. Linear function that correlates concentration of the aldehydic form of oleuropein aglycone (AOA) and sensory bitterness in the range 0.03 to 0.5 mmol/kg (*r,* linear regression coefficient; *P,* probability that $r = 0$).

bitterness of all varieties together, and even showed a non-significant correlation for Arbequina and Hojiblanca varieties considered separately (Table 3).

Whereas slopes for the equations obtained from the regressions between sensory bitterness and IB values obtained from different varieties vary from a minimum of 0.463 (Verdial) up to a maximum of 0.943 (Picual), the gradients obtained considering Equation 1 vary from a minimum of 0.951 (Picual) up to a maximum of 1.007 (Arbequina) only. This means that IB is effective for evaluating the bitterness of olive oils for samples obtained from each variety separately, but is less effective for comparing samples from different varieties. For this reason, Verdial oils showed higher IB values than Manzanilla oils, and the latter higher than Picual oils. All had similar levels of sensory-evaluated bitterness.

These results can be explained by the fact that AOA absorbs at 225 nm, together with other secoiridoid derivatives (DOA, DLA, and ALA) and other compounds of the phenolic extract that are not bitter (Fig. 2), such as the very abundant elenolic acid and other minor compounds (hydroxytyrosyl acetate, hydroxytyrosol, tyrosol, pinoresinol, and 1-acetoxypinoresinol).

ACKNOWLEDGMENTS

The authors are indebted to Rosario González-Cordones, María del Carmen Martínez, and Manuel Rodríguez-Aguilar for their technical assistance and to Comisión Interministerial de Ciencia y Tecnología (project OLI96-2159) and to Junta de Andalucía (project CAO 01-004) for its financial support. Furthermore, the authors would like to thank Francisco Ramos of Organización de Productores de Aceite de Oliva de Huelva, who provided the biological material, for his collaboration.

REFERENCES

1. Ruíz-Gutiérrez, V., F.Y.G. Muriana, and J. Villar, Virgin Olive Oil and Cardiovascular Diseases. Plasma Lipid Profile and Lipid Composition of Human Erythrocyte Membrane, *Grasas Aceites 49*:9–29 (1998).
2. Visioli, F., G. Bellomo, and C. Galli, Free Radical-Scavenging Properties of Olive Oil Polyphenols, *Biochem. Biophys. Res. Commun. 247*:60–64 (1998).

TABLE 3
Regression Obtained Between Sensory-Evaluated Bitterness (variable *y*) and Two Parameters Used for Estimating Olive Oil Bitterness

		Variable x = IB[a]			Variable x = Equation[b]		
Variety	n^c	R^2 [d]	$P_{(r=0)}$	Equation	R^2	$P_{(r=0)}$	Equation
Arbequina	6	0.5308	0.0630	$y = 0.603x$	0.9249	0.0005	$y = 1.007x$
Hojiblanca	3	0.5985	0.2264	$y = 0.532x$	0.9808	0.0096	$y = 0.971x$
Manzanilla	10	0.9858	0.0000	$y = 0.775x$	0.9882	0.0000	$y = 0.968x$
Picual	6	0.9350	0.0019	$y = 0.943x$	0.9846	0.0000	$y = 0.951x$
Verdial	14	0.9788	0.0000	$y = 0.463x$	0.9810	0.0000	$y = 0.995x$
All	39	0.8779	0.0000	$y = 0.584x$	0.9832	0.0000	$y = 0.975x$

[a]IB = 13.33 K_{225} – 0.837 (intensity of bitterness).
[b]x = 0.51 + 7.99 AOA, where AOA is expressed as mmol/kg.
[c]Number of samples.
[d]Multiple determination coefficient. For other abbreviations see Tables 1 and 2.

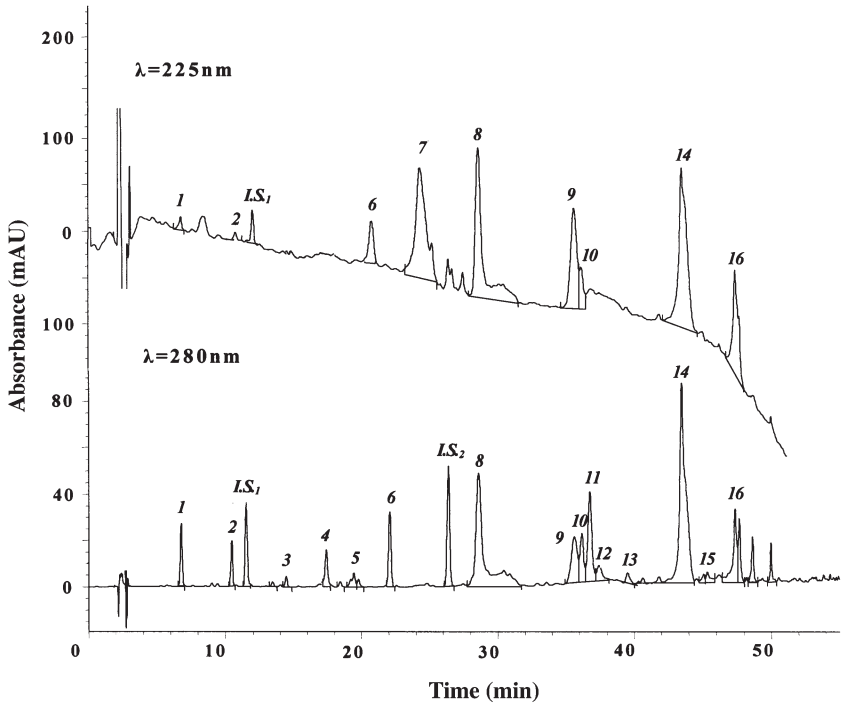

FIG. 2. HPLC chromatogram of the phenolic fraction obtained from Picual virgin olive oil by using solid-phase extraction with column packed with a diol-bonded phase and eluting with methanol, with detection at λ = 280 and 225 nm. Chromatographic peaks: (**1**) hydroxytyrosol; (**2**) tyrosol; (***IS*$_1$**) *p*-hydroxyphenylacetic acid; (**3**) vanillic acid; (**4**) vanillin; (**5**) *p*-coumaric acid; (**6**) hydroxytyrosyl acetate; (**7**) elenolic acid; (***IS*$_2$**) *o*-coumaric acid; (**8**) dialdehydic form of decarboxymethyl oleuropein aglycone; (**9**) dialdehydic form of decarboxymethyl ligstroside aglycone; (**10**) pinoresinol; (**11**) cinnamic acid; (**12**) 1-acetoxypinoresinol; (**13**) luteolin; (**14**) aldehydic form of oleuropein aglycone; (**15**) apigenin; (**16**) aldehydic form of ligstroside aglycone. *IS,* internal standard.

3. Visioli, F., and C. Galli, Olive Oil Phenols and Their Potential Effects on Human Health, *J. Agric. Food Chem. 46*:4292–4296 (1998).
4. European Commission Regulation EEC/2568/91 of 11 July on the Characteristics of Olive and Olive Pomace Oils and on Their Analytical Methods, *Off. J. Eur. Communities L 248*:1–82 (1991).
5. García, J.M., K. Yousfi, R. Mateos, M. Olmo, and A. Cert, Reduction of Oil Bitterness by Heating of Olive (*Olea europaea*) Fruits, *J. Agric. Food Chem. 49*:4231–4235 (2001).
6. Kiritsakis, A.K., Flavor Components of Olive Oil. A Review, *J. Am. Oil Chem. Soc*. *75*:673–681 (1998).
7. Soler-Rivas, C., J.C. Espin, and H.J. Wichers, Oleuropein and Related Compounds, *J. Sci. Food Agric. 80*:1013–1023 (2000).
8. Gutiérrez, F., M.A. Albi, R. Palma, J.J. Ríos, and J.M. Olías, Bitter Taste of Virgin Olive Oil: Correlation of Sensory Evaluation and Instrumental HPLC Analysis, *J. Food Sci. 54*:68–70 (1989).
9. Gutiérrez, F., S. Perdiguero, R. Gutiérrez, and J.M. Olías, Evaluation of the Bitter Taste in Virgin Olive Oil, *J. Am. Oil Chem. Soc*. *69*:394–395 (1992).
10. Baraldi, P.G., D. Simoni, S. Manfredini, and E. Menziani, Preparation of 3,4-Dihydroxy-1-benzeneethanol: A Reinvestigation, *Liebigs Ann. Chem.*:684–686 (1983).
11. Alcudia, F., A. Cert, J.L. Espartero, R. Mateos, and M. Trujillo, Procedure for Preparing Hydroxytyrosyl Esters, Spanish Patent 200,201,554 (2002).
12. Capasso, R., A. Evidente, C. Visca, L. Gianfreda, M. Maremonti, and G. Greco, Production of Glucose and Bioactive Aglycone by Chemical and Enzymatic Hydrolysis of Purified Oleuropein from *Olea europaea*, *Appl. Biochem. Biotechnol. 61*:365–377 (1996).
13. Montedoro, G., M. Servili, M. Baldioli, R. Selvaggini, E. Miniati, and A. Macchioni, Simple and Hydrolyzable Compounds in Virgin Olive Oil. 3. Spectroscopic Characterizations of the Secoiridoid Derivatives, *J. Agric. Food Chem. 41*:2228–2234 (1993).
14. Walter, W.M., Jr., H.P. Fleming, and J.L. Etchells, Preparation of Antimicrobial Compounds by Hydrolysis of Oleuropein from Green Olives, *Appl. Microbiol. 26*:773–776 (1973).
15. Mateos, R., J.L. Espartero, M. Trujillo, J.J. Ríos, M. León-Camacho, F. Alcudia, and A. Cert, Determination of Phenols, Flavones, and Lignans in Virgin Olive Oils by Solid-Phase Extraction and High-Performance Liquid Chromatography with Diode Array Ultraviolet Detection, *J. Agric. Food Chem. 49*:2185–2192 (2001).

[Received March 17, 2003; accepted October 13, 2003]