BITTER TASTE AND PHENOLIC COMPOUNDS IN EXTRA VIRGIN OLIVE OIL: AN EMPIRICAL RELATIONSHIP

SILVIA SILIANI[1,3], ALISSA MATTEI[2], LUCA BENEVIERI INNOCENTI[1] and BRUNO ZANONI[1]

[1]*Dipartimento di Biotecnologie Agrarie*
Sezione di Tecnologie Alimentari
Università degli Studi di Firenze
Via Donizetti, 6-50144 Firenze, Italia

[2]*Carapelli Firenze SpA*
Via Benvenuto Cellini
75-50028 Tavarnelle Val di Pesa ,Firenze, Italia

ABSTRACT

Bitter taste of extra virgin olive oil is known to be affected by the phenolic composition. However, contribution of each individual phenol to this sensory note has not been clearly defined. The aims of this study were to verify whether there was a relationship between bitter sensation and phenolic compound concentration, to determine which compounds were involved in bitter taste and to evaluate quantitatively this correlation. Results confirmed that a positive correlation did exist between total phenolic amount and bitter intensity. Data processing showed that this correlation was significantly dependent upon a relationship between oleuropein aglycon (3,4-DHPEA-EA) and bitter intensity. An empirical exponential model was set up and validated.

INTRODUCTION

Different classes of phenolic compounds can be found in extra virgin olive oil (EVOO). Some of them such as phenolic acid, hydroxy-isocromans, phenolic alcohols and flavonoids are present in small amounts, while some such as secoiridoids and lignans are present in large amounts (Tsimidou 1998; Brenes *et al*. 2000; Bianco *et al*. 2001; Servili *et al*. 2004). Sensory profile of EVOO is affected by the phenolic composition. A number of studies have shown that there is a correlation between bitter taste, astringency and pungency of EVOO, and total phenol concentration (Tsimidou 1998; Angerosa

[3] Corresponding author. TEL: (+39) 055-3220332; FAX: (+39) 055-355995; EMAIL: silvia.siliani@unifi.it

Journal of Food Quality **29** (2006) 431–441.

et al. 2000; Servili *et al*. 2004). Although it has been clearly defined that phenolic compounds are the main contributors to these characteristics, contribution of each individual phenol is not so clear. Secoiridoid derivatives seemed to play a significant role on sensory properties of EVOO. Some authors found a positive correlation between secoiridoid derivatives and EVOO bitterness and pungency (Garcia *et al*. 2001; Tovar *et al*. 2001; Andrewes *et al*. 2003; Gutierrez-Rosales *et al*. 2003). The following compounds have been taken into account in the said correlation: oleuropein aglycon (3,4-DHPEA-EA), dialdehydic form of decarboxymethyl oleuropein (3,4-DHPEA-EDA) and dialdehydic form of decarboxymethyl ligstroside (*p*-HPEA-EDA). Unfortunately, these correlations have limited operating utility, as they have rarely been defined by a kinetic relationship and they have not been validated statistically.

Recently, Mateos *et al*. (2004) found that none of the simple phenolic compounds of olive oil seemed to have a bitter taste, whereas among the secoiridoid derivatives of oleuropein aglycon showed a high intensity of this attribute. The aforementioned study reported a linear relationship between intensity of bitterness, measured by a panel test, and oleuropein aglycon concentration. Oleuropein aglycon concentration has been measured by HPLC and expressed as mmol/kg as compared to a standard aldehydic form of oleuropein aglycon, obtained from enzymatic hydrolysis of oleuropein by β-glucosidase.

The aims of this study were both to verify whether bitter sensation increased on increasing phenolic compound concentration, and to determine and validate empirical relationships between bitter intensity and phenolic composition in order to identify whether literature data could be confirmed or not.

MATERIALS AND METHODS

Materials

All virgin olive oil samples were purchased from Carapelli Firenze SpA (Florence, Italy). Two oil groups were used in this study. The first group (i.e., batch A) consisted of 18 virgin olive oil samples, which were purchased from different Mediterranean areas during the 2003 crop season and thus showed a range of slight to extreme bitter taste. These samples were used to assess a relationship between bitterness and phenolic composition. The second group (i.e., batch B) consisted of 23 virgin olive oil samples, which were purchased not only from Mediterranean areas but also from other regions (e.g., Australia) during different crop seasons. These oil samples were used to validate the kinetics developed.

TABLE 1.
GRADIENT ELUTION PROGRAM

Time (min)	Water (%)	Methanol (%)	Acetonitrile (%)
0	96	2	2
40	50	25	25
45	40	30	30
60	0	50	50
70	0	50	50
72	96	2	2
82	96	2	2

Chemical Analyses

Acidity (% oleic acid), fatty acid content (%), peroxide value (meqO_2/kg) and spectroscopic parameters, i.e., K_{232} and K_{270}, in the UV region were measured by European Union official methods (Anon 2002).

Secoiridoid and lignan concentration was determined by HPLC following the method described by Cortesi *et al*. (2002) with some modifications. Oil (2 g ± 0.1 g) was dissolved in 1 mL of hexane and stirred on vortex for 30 s; 1 mL of internal standard (syringic acid in methanol 80% v/v) was added, and the solution was stirred. After centrifugation (15 min, 4000 rpm), the subnatant fraction was taken out, transferred to a screw cone tube and purified with 1 mL of hexane; it was stirred on vortex for 1 min and centrifuged (7 min, 4000 rpm). The subnatant fraction was taken out, and 20 µL of sample was injected in HPLC.

The HPLC apparatus consisted of a vacuum degasser (Spectra System SMC1000 (Thermo Electron Corporation, Waltham, MA)), a quaternary pump (Spectra System P4000 (Thermo Electron Corporation)), a column (Alltech Spherisorb ODS-2 RP18 (Alltech Associates Inc., Deerfield, IL)) and a diode array detector (Spectra System UV6000 LP (Thermo Electron Corporation)). Elution was carried out at a flow rate of 1 mL/min using a water, methanol and acetonitrile mixture as a mobile phase (Table 1).

Chromatograms were acquired at 280 nm. They were recorded and processed using ChromQuest Chromatography software (Thermo Electron Corporation). Phenolic and lignan compounds were identified (Table 2) and quantified (ppm) as a tyrosol equivalent by the ratio of syringic acid response factor to tyrosol response factor (Cortesi *et al*. 2002). The total phenolic compound content (ppm) was determined by the sum of individual, either identified or not identified, chromatographic peaks.

Sensory Analysis

Sensory evaluation was performed by five judges, who were familiar with oil sensory quality, according to the EU official method (Anon 2002). Judges

TABLE 2.
PHENOLIC COMPOUNDS DETECTED AS A FUNCTION OF ELUTION ORDER

Hydroxytyrosol (3,4-DHPEA)
Tyrosol (*p*-HPEA)
Dialdehydic form of decarboxymethyl oleuropein aglycon (3,4-DHPEA-EDA)
Dialdehydic form of oleuropein aglycon
Dialdehydic form of decarboxymethyl ligstroside aglycon (*p*-HPEA-EDA)
Dialdehydic form of ligstroside aglycon
Oleuropein aglycon (3,4-DHPEA-EA)
Ligstroside aglycon (*p*-HPEA-EA)

were requested to evaluate the intensity of bitter taste by assigning a score between 0 (absence of attribute) and 5 (extreme intensity of attribute).

Data Processing

Linear and nonlinear regression analyses were carried out by TableCurve (Jandel Scientific Software, Erkrath, Germany).

RESULTS AND DISCUSSION

All oil samples were proved to be EVOO by chemical and sensory analyses in compliance with EU regulations (Anon 2002).

Table 3 shows the phenolic and lignan compound concentration of EVOO from batch A as a function of bitter intensity. Data showed that an increase in bitter intensity reflected an increase in the total phenolic compound content. Figure 1 clearly shows that complexity of chromatograms increased on increasing bitter intensity.

Concentrations of secoiridoids and lignans were processed as a function of bitter intensity by both linear and nonlinear regression analyses. A statistically significant linear relationship was found between total phenolic amount and bitterness. Several regression analyses were carried out to identify which single compound or sum of compounds better explained this relationship, that is to find those relationships that maximized the correlation coefficient between bitterness and concentration of individual phenolic compounds. The highest statistical significance was obtained from the exponential relationship between oleuropein aglycon (3,4-DHPEA-EA) and bitterness.

Table 4 shows the two aforementioned empirical relationships with relevant constants and statistical numeric summaries; Figs. 2 and 3 show the relevant regression graphs.

Relationships were validated by EVOO from batch B. The large number of EVOO samples and the absence of correlation between them in terms of

TABLE 3.
PHENOLICS AND LIGNAN COMPOUNDS CONCENTRATION (PPM) OF EXTRA VIRGIN OLIVE OIL FROM BATCH A AS A FUNCTION OF BITTER INTENSITY

Bitter intensity	0	1			2			3		3.5		4						
Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
3,4-DHPEA	6.48	12.28	10.44	16.16	1.91	20.12	4.80	8.43	1.94	10.92	13.29	15.49	3.46	15.13	8.30	12.94	5.00	12.00
p-HPEA	8.29	17.56	13.12	20.65	8.15	8.31	4.56	7.18	3.12	13.38	8.16	7.52	5.21	12.69	4.93	6.87	6.55	5.41
3,4-DHPEA-EDA	1.04	8.77	9.49	11.13	7.18	13.71	79.37	81.30	60.17	59.83	139.64	66.07	126.91	77.52	82.18	114.00	59.69	107.77
Dialdehydic form of oleuropein aglycon	1.54	3.06	2.71	3.57	1.03	1.60	1.92	6.94	0.00	4.47	8.20	5.76	10.08	4.23	9.57	8.44	2.43	9.24
p-HPEA-EDA	5.99	17.17	15.77	21.46	74.12	10.68	44.54	58.21	36.58	107.40	107.01	58.02	89.60	54.67	64.99	67.48	65.52	50.94
Lignans	3.61	5.98	6.34	8.52	9.46	10.86	10.82	14.25	15.99	10.70	11.75	14.74	19.34	18.97	15.70	14.79	10.96	14.40
Dialdehydic form of ligstroside aglycon	8.43	14.05	15.74	17.63	36.04	4.66	21.39	13.50	35.83	42.40	12.73	18.40	31.21	15.47	20.79	17.79	6.93	11.16
3,4-DHPEA-EA	5.61	20.33	21.18	26.03	14.37	39.37	26.83	96.59	67.58	65.62	63.35	136.85	55.63	136.56	116.21	130.59	157.45	126.42
p-HPEA-EA	2.31	7.21	7.48	10.10	19.14	8.35	3.56	23.88	12.33	31.23	24.33	35.36	17.45	38.47	29.85	36.44	55.79	25.42
Total	79.84	147.68	150.07	179.58	234.14	192.12	256.94	391.33	322.95	410.72	436.58	459.15	451.47	444.04	468.62	500.67	421.83	490.44

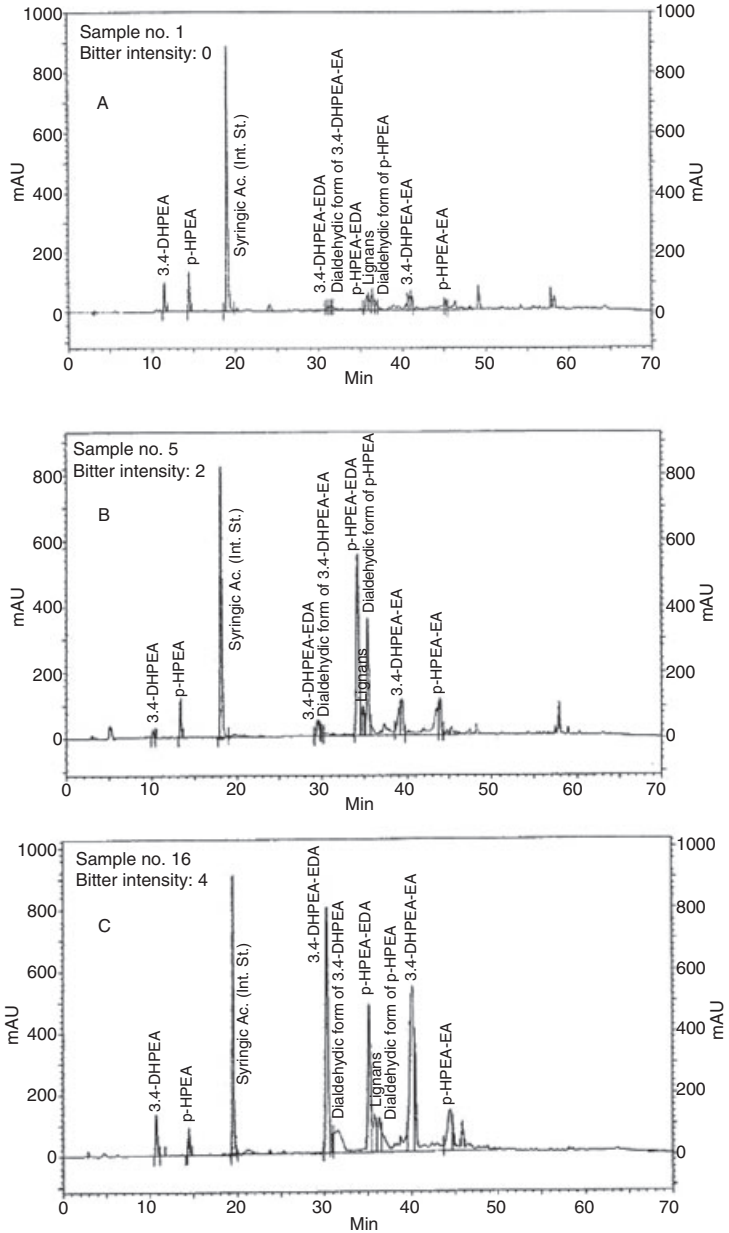

FIG. 1. CHROMATOGRAMS FOR DIFFERENT EVOO SAMPLES: A FOR SAMPLE NO. 1; B FOR SAMPLE NO. 5; C FOR SAMPLE NO. 16

TABLE 4.
EMPIRICAL RELATIONSHIPS BETWEEN TOTAL PHENOLIC COMPOUNDS CONCENTRATION, OLEUROPEIN AGLYCON (3,4-DHPEA-EA) AND BITTER INTENSITY WITH RELEVANT CONSTANTS AND STATISTICAL NUMERIC SUMMARIES

Parameters	Empirical relationship	r	P-value	Constants	95% confidence limits of constants	
Total phenolic compounds (ppm) versus bitter intensity	$y = a + b \cdot x$	0.98	$<10^{-6}$	$a = 51.94$	17.66	86.21
				$b = 102.06$	90.89	113.22
Oleuropein aglycon (ppm) versus bitter intensity	$y = a \cdot \exp(b \cdot x)$	0.95	$<10^{-6}$	$a = 7.46$	1.27	13.65
				$b = 0.716$	0.499	0.932

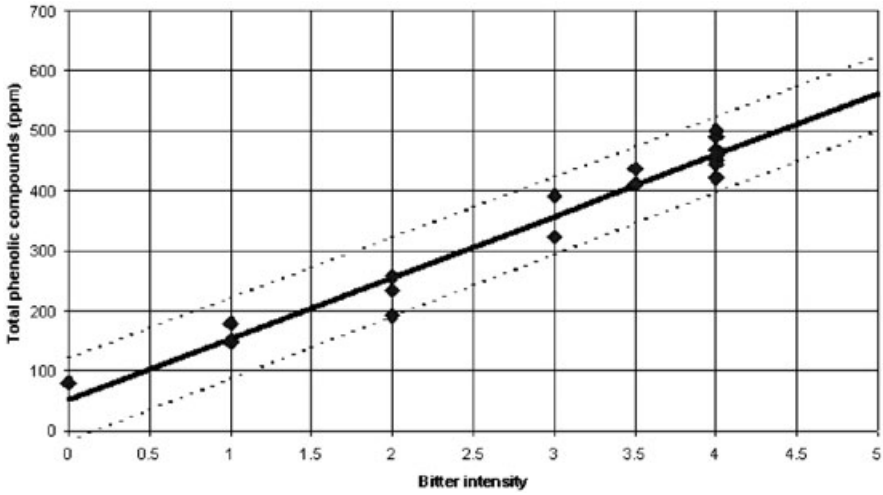

FIG. 2. RELATIONSHIP BETWEEN TOTAL PHENOLIC COMPOUNDS AND BITTER INTENSITY. SYMBOLS ARE EXPERIMENTAL DATA FOR EVOO FROM BATCH A; A CONTINUOUS LINE REPRESENTS THE EMPIRICAL LINEAR REGRESSION MODEL; DOTTED LINES REPRESENT 95% OF PREDICTION LIMITS FOR THE REGRESSION MODEL

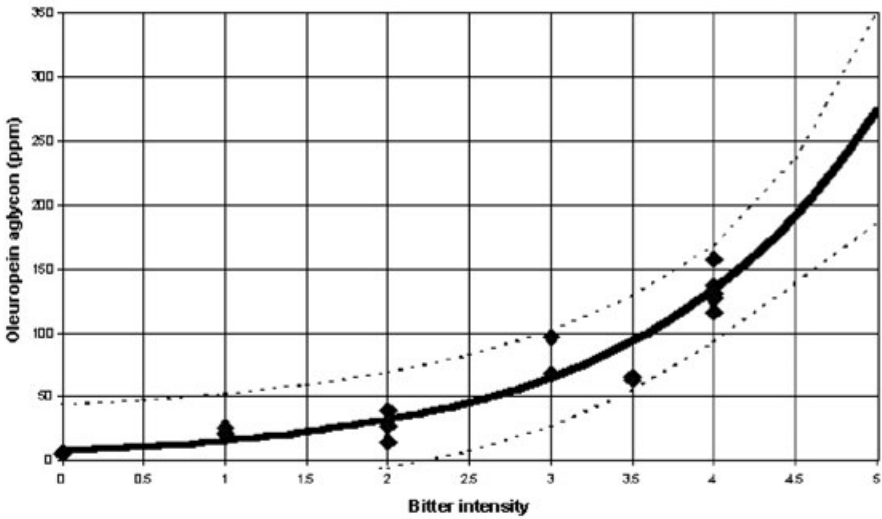

FIG. 3. RELATIONSHIP BETWEEN OLEUROPEIN AGLYCON (3,4-DHPEA-EA) AND BITTER INTENSITY. SYMBOLS ARE EXPERIMENTAL DATA FOR EVOO FROM BATCH A; A CONTINUOUS LINE REPRESENTS THE EMPIRICAL EXPONENTIAL REGRESSION MODEL; DOTTED LINES REPRESENT 95% OF PREDICTION LIMITS FOR THE REGRESSION MODEL

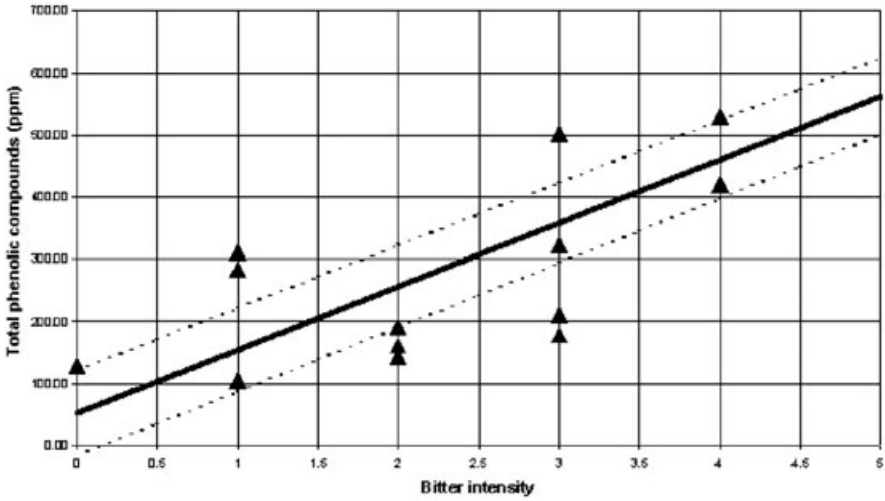

FIG. 4. RELATIONSHIP BETWEEN OLEUROPEIN AGLYCON (3,4-DHPEA-EA) AND BITTER INTENSITY. SYMBOLS ARE EXPERIMENTAL DATA FOR EVOO FROM BATCH A; A CONTINUOUS LINE REPRESENTS THE EMPIRICAL EXPONENTIAL REGRESSION MODEL; DOTTED LINES REPRESENT 95% OF PREDICTION LIMITS FOR THE REGRESSION MODEL

origin, crop season and extraction conditions allowed us to carry out a wide validation. Unfortunately, it was not possible to purchase EVOO with extreme bitter intensity (e.g., 5).

Results from validation are shown in Figs. 4 and 5. It can be seen that the relationship between oleuropein aglycon (3,4-DHPEA-EA) and bitter intensity was the only statistically validated relationship; most experimental data on EVOO from batch B were found to be within prediction limits of the regression model.

Our results were in agreement with those found by Mateos *et al*. (2004), but they were not in agreement with those reported by Gutierrez-Rosales *et al*. (2003). As compared to the study of Mateos *et al*. (2004), our empirical relationship between bitter taste and oleuropein aglycon showed a different trend and it was subjected to a strict statistical validation, as reported earlier.

In conclusion, this study showed that a positive correlation does exist between the amount of phenolic compounds and bitter intensity. This correlation was significantly due to a relationship between oleuropein aglycon (3,4-DHPEA-EA) and bitter intensity; an empirical exponential model was set up and validated.

This model may be useful for olive oil mill companies, which may not be provided with equipped laboratories, but they may be able to perform sensory

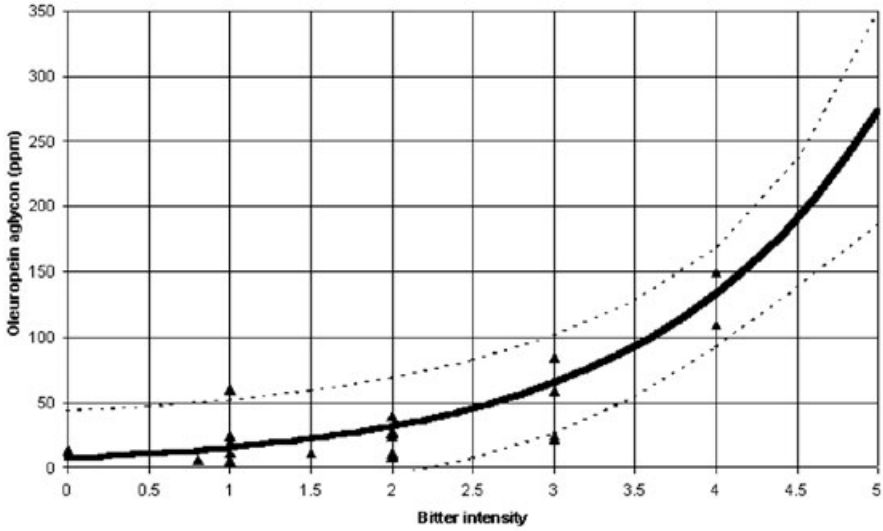

FIG. 5. VALIDATION OF THE RELATIONSHIP BETWEEN OLEUROPEIN AGLYCON (3,4-DHPEA-EA) AND BITTER INTENSITY. SYMBOLS ARE EXPERIMENTAL DATA FOR EVOO FROM BATCH B; A CONTINUOUS LINE REPRESENTS THE EMPIRICAL EXPONENTIAL REGRESSION MODEL; DOTTED LINES REPRESENT 95% OF PREDICTION LIMITS FOR THE REGRESSION MODEL

determinations of bitterness and may predict the phenolic content indirectly on the basis of bitterness and, hence, the nutritional power of oil. This model may also be useful for oil-blending companies, for which the foregoing results may apply. Most interestingly, they may better check their sensory panel's actions.

REFERENCES

ANDREWES, P., BUSCH, J.L.H.C., DE JOODE, T., GROENEWEGEN, A. and ALEXANDRE, H. 2003. Sensory properties of virgin olive oil polyphenols: Identification of deacetoxy-ligstroside aglycon as a key contributor to pungency. J. Agric. Food Chem. *51*, 1415–1420.

ANGEROSA, F., MOSTALLINO, R., BASTI, C. and VITO, R. 2000. Virgin olive oil odour notes: Their relationship with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. Food Chem. *68*, 283–287.

ANONYMOUS. 2002. Council Regulation (EC) no. 796/2002. Off. J. Comm. E.C. *L128*(May*15*), 1–28.

BIANCO, A., COCCIOLI, F., GUISO, M. and MARRAM, C. 2001. The occurrence in olive oil of a new class of phenolic compounds: Hydroxy-isocromans. Food Chem. *77*, 405–411.

BRENES, M., HIDALGO, F.J., GARCIA, A., RIOS, J.J., GARCIA, P., ZAMORA, R. and GARRIDO, A. 2000. Pinoresinol and 1-acetoxypinoresinol, two new phenolic compounds identified in olive oil. J. Am. Oil Chem. Soc. *77*, 715–720.

CORTESI, N., ROVELLINI, P. and FUSARI, P. 2002. Dosaggio dei biofenoli degli oli vergini di oliva: idrossitirosolo e tirosolo, agliconi secoiridoidi, acidi secoiridoidi, lignani e flavonoidi. Riv. Ital. Sost. Grasse *79*, 145–150.

GARCIA, J.M., YOUSFI, K., MATEOS, R., OLMO, M. and CERT, A. 2001. Reduction of oil bitterness by heathing of olive (*Olea europaea*) fruits. J. Agric. Food Chem. *49*, 4231–4235.

GUTIERREZ-ROSALES, F., RIOS, J.J. and GOMEZ-REY, M.L. 2003. Main polyphenols in the bitter taste of virgin olive oil: Structural confirmation by on-line high-performance liquid chromatography electrospray ionization mass spectrometry. J. Agric. Food Chem. *51*, 6021–6025.

MATEOS, R., CERT, A., PÉREZ-CAMINO, M.C. and GARCIA, M.J. 2004. Evaluation of virgin olive oil bitterness by quantification of secoiridoid derivatives. J. Am. Oil Chem. Soc. *81*, 71–75.

SERVILI, M., SELVAGGINI, R., ESPOSTO, S., TATICCHI, A., MONTEDORO, G.F. and MOROZZI, G. 2004. Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic and technological aspects of production that affect their occurrence in the oil. J. Chromatogr. A *1054*, 113–127.

TOVAR, M.J., MOTILVA, M.J. and ROMERO, M.P. 2001. Changes in the phenolic composition of virgin olive oil from young trees (*Olea europaea* L. cv. *Arbequina*) grown under linear irrigation strategies. J. Agric. Food Chem. *49*, 5502–5508.

TSIMIDOU, M. 1998. Polyphenols and quality of virgin olive oil in retrospect. Ital. J. Food Sci. *2*, 99–116.