Food Chemistry 112 (2009) 525–532

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Minor compounds in the phenolic fraction of virgin olive oils

Marcello Saitta *, Francesco Salvo, Giuseppa Di Bella, Giacomo Dugo, Giovanna Loredana La Torre

Dipartimento di Scienze degli Alimenti e dell'Ambiente, Universita' di Messina, Salita Sperone 31, 98166 Messina, Italy

ARTICLE INFO

Article history:
Received 27 March 2008
Received in revised form 2 May 2008
Accepted 3 June 2008

Keywords:
Gas chromatography
Mass spectrometry
Olive oils
Phenols

ABSTRACT

The phenolic fraction of 34 virgin olive oils was analysed by gas chromatography–mass spectrometry (GC–MS) with the aim to identify new compounds at low level. Twenty-seven compounds previously described in olive oils were identified; several new minor compounds with phenolic structure were detected in the samples: amongst them, 4-hydroxyphenylacetaldehyde, *trans*-isoeugenol (*trans*-2-methoxy-4-(1-propenyl)-phenol), 1,4-dihydroxy-2,6-dimethoxybenzene, 3,4-dihydroxybenzyl alcohol and 3,4-dihydroxyphenylacetic acid were identified by their mass spectra and confirmed using standards. In 34 virgin olive oils (cv. *Nocellara del Belice*) the mean concentrations for these five substances were always below 0.2 mg kg^{-1} and only in two samples the level of 3,4-dihydroxyphenylacetic acid reached 1.47 and 1.97 mg kg^{-1}, respectively. These compounds could be used to characterise olive oils; low concentrations of 3,4-dihydroxyphenylacetic acid may show the initial autoxidation processes of olive oils.
© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

The phenolic compounds are natural antioxidants present in virgin olive oils (Baldioli, Servili, Perretti, & Montedoro, 1996; Montedoro & Cantarelli 1969; Montedoro, 1972; Papadopoulos & Boskou, 1991; Vazquez Roncero, Janer del Valle, & Janer del Valle, 1975) but not in refined ones (Montedoro & Cantarelli, 1969). The importance of these compounds is due to the protective effect against colorectal and breast cancer and heart disease (Gerber, 1994; Owen, Mier, Giacosa, Hull, Spiegelhalder, & Bartsch, 2000). Their amount is related to olive variety and ripeness, climate, location, type of crushing machine and oil extraction procedures (Amiot, Fleuriet, & Macheix, 1986; Catalano & Caponio, 1996; Solinas, Di Giovacchino, & Mascolo, 1978). The presence of phenols is also responsible for the bitterness and the pungency of the olive oils (Andrewes, Busch, de Joode, Groenewegen, & Alexandre, 2003; Gutierrez-Rosales, Rios, & Gomez-Rey, 2003). The analyses of phenols are carried out by gas chromatography (Angerosa, d'Alessandro, Konstantinou, & Di Giacinto, 1995; Saitta, Lo Curto, Salvo, Di Bella, & Dugo, 2002) or by high performance liquid chromatography (HPLC) (Akasbi, Shoeman, & Saari Csallany, 1993; Bianco, Mazzei, Melchioni, Romeo, Scarpati, Soriero, & Uccella, 1998; Brenes, Garcia, Garcia, Rios, & Garrido, 1999; Montedoro, Servili, Baldioli, & Miniati, 1992a, 1992b); in the last few years, researches have been conducted on monocultivar samples to characterise them (Garcia, Brenes, Romero, Garcia, & Garrido, 2002; Gomez-Alonso, Salvador, & Fregapane, 2002; Pinelli, Galardi, Mulinacci, Vincieri, Cimato, & Romani, 2003) and new compounds were identified (Bianco, Coccioli, Guiso, & Marra, 2001; Brenes et al., 1999; Saitta et al., 2002).

The GC technique has the advantages of lower detection limits and better separations compared to HPLC; GC–MS enables also a direct qualitative information based on the mass spectra: it is undoubtly the simplest way to identify free phenols. In this investigation, we studied by GC–MS 34 monovarietal samples of olive oils (cv. *Nocellara del Belice*) produced in Sicily in the year 2003, with the aim to identify new phenols at low levels (<0.1 mg kg^{-1}). Minor compounds can help to discriminate oils from different varieties and origins, simplifying the controls against oil adulterations; some components could be related to an initial oil oxidation process.

2. Materials and methods

2.1. Virgin olive oils

Commercially available Sicilian oils extracted from olives of *Nocellara del Belice* variety (34 samples) were used. All samples were kept at 5 °C under nitrogen until analysis.

2.2. Chemicals and materials

Solvents and standard phenols were obtained from Sigma–Aldrich (Milwaukee, WI). 4-Hydroxyphenylacetaldehyde was synthesised according to the procedure of Parikh and von Doering (1967). 3,4-Dihydroxybenzyl alcohol was synthesised according to the procedure of Pyne, Truscott, Maxwell, Morales, Walsh, and Wynn (1990). A dichloromethane solution of sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid) was prepared weekly at the

* Corresponding author. Tel.: +39 90 6765181; fax: +39 90 6765436.
E-mail address: msaitta@unime.it (M. Saitta).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.06.001

concentration of 500 mg/L and stored at 5 °C. Bis(trimethylsilyl)trifluoroacetamide: trimethylchlorosilane (BSTFA:TMCS, 99:1) was purchased from Supelco (Bellefonte, PA).

2.3. Extraction of phenolic compounds

Extraction was carried out as follows: 5 g of oil were added of a known amount of sinapinic acid (internal standard) and extracted four times with 5 mL of methanol/water (80:20 v/v), then the solution was subjected to a stream of nitrogen to remove the methanol. The residue was taken up with 5 mL of acetonitrile and the solution was washed three times with 5 mL of *n*-hexane to eliminate small amounts of triglycerides.

2.4. GC–MS analysis

One millilitre of acetonitrile solution was evaporated using a stream of nitrogen; the residue was derivatized with 200 μL of BSTFA-TMCS (99:1) at room temperature for 30 min to prepare the trimethylsilyl (TMS) derivatives. Analysis was carried out with a Finnigan MAT (San Jose, CA) GCQ GC–MS system equipped with a split-splitless injector and a Restek (Bellefonte, PA) RTX-5MS column (30 m, 0.25 mm i.d., 0.25 μm film thickness). The oven temperature was programmed as follows: from 60 to 150 °C at 15 °C/min, from 150 to 275 °C/min at 5 °C/min and then isothermal for further 24 min. Transfer-line was set at 275 °C, injector at 250 °C and ion source at 200 °C. Injections were performed with a 60 s splitless and the carrier gas was Helium, at 40 cm/s (constant flow). The mass spectrometer was used in full scan EI (70 eV) mode, from 40 to 800 Da, 1 spec/s. The detected compounds are reported in Table 1; identifications were confirmed using standard or synthesised compounds to compare spectra and retention times. Quantitation was performed in ion current using base and molecular peaks (if the ions were coincident, the two most intense peaks were chosen); sinapinic acid (retention time: 22′48″) was used as internal standard.

3. Results and discussion

3.1. Considerations about the phenolic compounds and the TMS derivatives

The chromatogram of the phenolic fraction of an olive oil is quite complex and some interferences (fatty acids, monoglycerides) are present. Fig. 1 shows part of a chromatogram of a *Nocellara del Belice* sample. The phenolic compounds are numerous: their characteristic mass spectra enable the identification of 27 known phenols (Table 1); decarbomethoxy elenolic acid (dialdehydic form) linked to homovanillic alcohol was not confirmed because there was not a suitable standard. In these compounds (aromatic alcohols, aldehydes, acids or esters), the first phenolic hydroxy group is almost all in the *para* position, and a methoxy group, if present, is in the *meta* position. The most abundant compounds were β-phenylethyl alcohols like tyrosol (4-hydroxyphenyl ethanol), hydroxytyrosol (3,4-dihydroxyphenyl ethanol) and their esters with elenolic acid, decarbomethoxy ligstroside and oleuropein aglycons; unlike previously reported in Sicilian olive oils (Saitta et al., 2002), we found both carbomethoxy and decarbomethoxy aglycons in our samples (Table 2).

The TMS derivatives are very useful in this kind of analysis, because they make easy the discrimination of the phenols; common losses are 15 Da (methyl group), 29 Da (–CHO from alkylic aldehydes), 30 Da (HCHO from methoxyphenyl derivatives), 89 Da (–OTMS), 103 Da (–CH_2OTMS from phenylethyl and benzyl derivatives) and typical intense fragments are at *m/z* 179 $[TMSOC_6H_4CH_2]^+$ and 267 $[(TMSO)_2C_6H_3CH_2]^+$.

Table 1
Compounds detected, retention times, molecular weights and base peaks in the mass spectra of the TMS derivatives

Compound	RT (min)	MW	bp
(A) 4-Hydroxyphenylacetaldehyde	8′31″	208	179
(1) Vanillin (3-methoxy-4-hydroxybenzaldehyde)	10′16″	224	194
(2) Tyrosol (4-hydroxyphenylethanol)	10′40″	282	179
(B) *trans*-Isoeugenol (*trans*-2-methoxy-4-(1-propenyl)-phenol)	10′42″	236	206
(3) 4-(Acetoxyethyl)-1-hydroxybenzene	11′27″	252	192
(4) 4-Hydroxybenzoic acid	11′35″	282	267
(5) Vanillic alcohol (3-methoxy-4-hydroxybenzyl alcohol)	11′53″	298	298
(6) 3,4-Dihydroxyphenylacetaldehyde	11′57″	296	267
(C) 1,4-Dihydroxy-2,6-dimethoxybenzene	12′36″	314	314
(D) 3,4-Dihydroxybenzyl alcohol	12′57″	356	179
(7) Homovanillic alcohol (3-methoxy-4-hydroxyphenylethanol)	13′07″	312	312
(8) Syringaldehyde (3,5-dimethoxy-4-hydroxybenzaldehyde)	13′09″	254	224
(9) Vanillic acid (3-methoxy-4-hydroxybenzoic acid)	14′07″	312	297
(10) Hydroxytyrosol (3,4-dihydroxyphenylethanol)	14′09″	370	267
(11) 2-Coumaric acid (*trans*-2-hydroxycinnamic acid)	14′33″	308	293
(12) 4-(Acetoxyethyl)-1,2-dihydroxybenzene	15′07″	340	280
(13) Protocatechuic acid (3,4-dihydroxybenzoic acid)	15′09″	370	193
(E) 3,4-Dihydroxyphenylacetic acid	15′18″	384	179
(14) Syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid)	16′37″	342	327
(15) *cis*-Ferulic acid (*cis*-3-methoxy-4-hydroxycinnamic acid)	17′03″	338	338
(16) 4-Coumaric acid (*trans*-4-hydroxycinnamic acid)	17′18″	308	293
(17) Ferulic acid (*trans*-3-methoxy-4-hydroxycinnamic acid)	20′08″	338	338
(18) Caffeic acid (*trans*-3,4-dihydroxycinnamic acid)	20′52″	396	219
(19) Decarbomethoxy ligstroside aglicon (dialdehydic form)	27′44″	376	192
(20) Decarbomethoxy ligstroside aglicon	28′43″	448	192
(21) Decarbomethoxy elenolic acid (dialdehydic form) linked to homovanillic alcohol[a]	29′46″	406	192
(22) Decarbomethoxy oleuropein aglycon (dialdehydic form)	30′24″	464	280
(23) Decarbomethoxy oleuropein aglycon	31′40″	536	280
(24) Ligstroside aglicon (dialdehydic form)	31′50″	434	192
(25) Ligstroside aglicon	34′03″	506	192
(26) Oleuropein aglycon (dialdehydic form)	34′53″	522	280
(27) Oleuropein aglycon	37′34″	594	280

[a] Not confirmed.

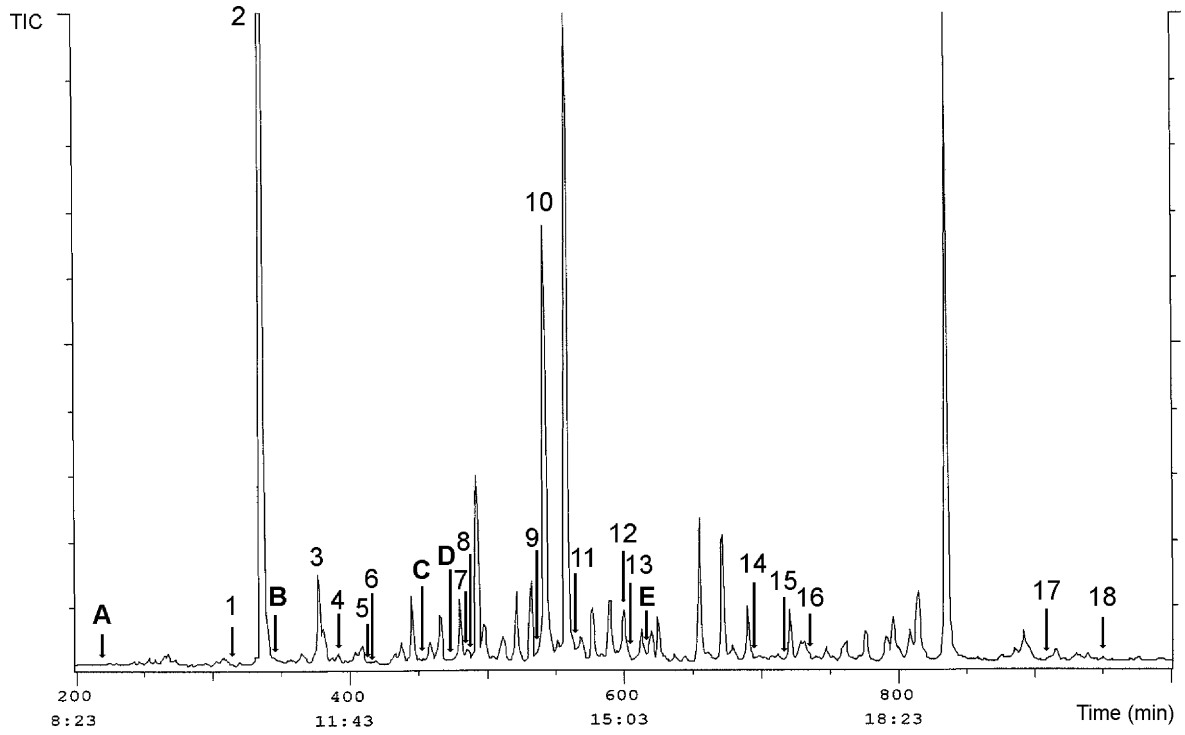

Fig. 1. Part of a chromatogram of the phenolic compounds (TMS derivatives) of a *Nocellara del Belice* olive oil sample (Peaks identification as in Table 1).

Table 2
Phenols amount (mg kg^{-1}) in 34 samples of olive oils (cv *Nocellara del Belice*)

Compound	Range	Mean ± SD
(A) 4-Hydroxyphenylacetaldehyde	0.011–0.076	0.034 ± 0.016
(1) Vanillin (3-methoxy-4-hydroxybenzaldehyde)	0.12–0.26	0.19 ± 0.04
(2) Tyrosol (4-hydroxyphenylethanol)	10.5–32.3	19.3 ± 5.3
(B) *trans*-Isoeugenol (*trans*-2-methoxy-4-(1-propenyl)-phenol)	<0.002–0.077	0.026 ± 0.017
(3) 4-(Acetoxyethyl)-1-hydroxybenzene	2.22–6.03	3.72 ± 1.01
(4) 4-Hydroxybenzoic acid	0.045–0.28	0.10 ± 0.05
(5) Vanillic alcohol (3-methoxy-4-hydroxybenzyl alcohol)	0.023–0.17	0.061 ± 0.038
(6) 3,4-Dihydroxyphenylacetaldehyde	0.18–0.65	0.35 ± 0.12
(C) 1,4-Dihydroxy-2,6-dimethoxybenzene	<0.002–0.028	0.005 ± 0.006
(D) 3,4-Dihydroxybenzyl alcohol	<0.002–0.31	0.023 ± 0.052
(7) Homovanillic alcohol (3-methoxy-4-hydroxyphenylethanol)	0.78–1.36	0.99 ± 0.18
(8) Syringaldehyde (3,5-dimethoxy-4-hydroxybenzaldehyde)	0.037–0.29	0.087 ± 0.054
(9) Vanillic acid (3-methoxy-4-hydroxybenzoic acid)	0.93–2.94	1.60 ± 0.44
(10) Hydroxytyrosol (3,4-dihydroxyphenylethanol)	12.8–36.1	25.4 ± 6.4
(11) 2-Coumaric acid (*trans*-2-hydroxycinnamic acid)	0.018–0.20	0.076 ± 0.039
(12) 4-(Acetoxyethyl)-1,2-dihydroxybenzene	3.59–8.26	5.64 ± 1.23
(13) Protocatechuic acid (3,4-dihydroxybenzoic acid)	<0.002–0.25	0.088 ± 0.061
(E) 3,4-Dihydroxyphenylacetic acid	<0.002–1.97	0.17 ± 0.34
(14) Syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid)	0.014–0.15	0.068 ± 0.031
(15) *cis*-Ferulic acid (*cis*-3-methoxy-4-hydroxycinnamic acid)	<0.002–0.17	0.070 ± 0.051
(16) 4-Coumaric acid (*trans*-4-hydroxycinnamic acid)	0.67–1.68	1.14 ± 0.28
(17) Ferulic acid (*trans*-3-methoxy-4-hydroxycinnamic acid)	0.29–0.72	0.47 ± 0.12
(18) Caffeic acid (*trans*-3,4-dihydroxycinnamic acid)	0.078–0.92	0.42 ± 0.27
(19) Decarbomethoxy ligstroside aglicon (dialdehydic form)	82.4–156	115 ± 20
(20) Decarbomethoxy ligstroside aglicon	2.25–8.53	4.62 ± 1.90
(21) Decarbomethoxy elenolic acid (dialdehydic form) linked to homovanillic alcohol[a]	0.75–1.92	1.20 ± 0.37
(22) Decarbomethoxy oleuropein aglycon (dialdehydic form)	66.8–124	95.6 ± 15.7
(23) Decarbomethoxy oleuropein aglycon	1.73–7.89	3.13 ± 1.34
(24) Ligstroside aglicon (dialdehydic form)	0.78–2.25	1.24 ± 0.41
(25) Ligstroside aglicon	1.35–3.58	2.09 ± 0.57
(26) Oleuropein aglycon (dialdehydic form)	0.86–1.94	1.19 ± 0.30
(27) Oleuropein aglycon	1.54–4.28	2.49 ± 0.73

[a] Not confirmed.

In our reconstructed chromatograms, the total ionic current (TIC) is composed of about 2600 EI spectra. Small amounts of phenols (<0.1 mg kg^{-1}) cannot be simply detected as peaks in a reconstructed chromatogram; to evaluate the presence of unknown phenols in the olive oils, thousands of spectra were examined, chosen and compared amongst the samples; only the spectra showing characteristic fragments and losses were considered. In this way, several possible new phenols were selected: five new phenols were

identified in this way for the first time in olive oil samples. Quantitative data were obtained from selected ions chromatograms extracted from the TIC traces.

3.2. Identification of the structures

The first unknown compound in retention time order (marked as A in Fig. 1) was detected in all the 34 samples. The mass spectrum was very simple and clean: base peak was at *m/z* 179 and the molecular ion was at *m/z* 208 (Fig. 2). The difference between the ions is 29 Da and a logical structure for this compound could be 4-hydroxyphenylacetaldehyde. This compound was synthesised, derivatized and analysed: spectrum and retention time were the same of compound A. The mean amount of 4-hydroxyphenylacetaldehyde was 0.034 mg kg^{-1} (Table 2).

The second unknown compound, B, is not easily detectable. It had a retention time very close to that of tyrosol and was ever hidden by it. In Fig. 3 are reported two consecutive spectra showing clean tyrosol and tyrosol + compound B, respectively: the differences are in the ions at *m/z* 236, 221 and 206. If B was a phenol, there should be a silylated –OH and a methoxy group on a benzene ring [(TMSO)(CH_3O)C_6H_3–]: this structure can justify 195 Da on a molecular weight of 236. The difference (41 Da) could be only an allyl group, –CH=CH–CH_3 or –CH_2–CH=CH_2. Compound B should have the hydroxy group in *para* and the methoxy one in *meta* with respect to the allyl group, so there were two possibilities: eugenol (2-methoxy-4-(2-propenyl)-phenol) and isoeugenol (*trans*-2-methoxy-4-(1-propenyl)-phenol). These compounds were commercially available and the TMS derivatives were analysed; the spectra showed three major ions at *m/z* 206 (base peak), 221 and 236 (molecular ion). The retention times were as follows: eugenol 9′16″, *cis*-isoeugenol (impurity in the standard of the *trans*-isoeugenol) 10′01″, *trans*-isoeugenol 10′42″; compound B had a retention time of 10′42″ (Table 1) and the structure of *trans*-isoeugenol can be assigned. This structure is related to those of cinnamic acids. In Fig. 4 the spectrum of *trans*-isoeugenol (TMS der.) is shown. The mean amount of *trans*-isoeugenol was 0.026 mg kg^{-1} (Table 2). In Fig. 4 is also reported the chromatogram (TIC and selected ions) of an olive oil sample showing the peak corresponding to *trans*-isoeugenol at the *m/z* values of 206 and 236.

Compound C was present in very low content. In the spectrum of C only two ions were abundant, at *m/z* 314 (base peak and molecular ion) and 284; small ions were present at *m/z* 299, 269 and 254 (Fig. 5A). Compound C could have two methoxy groups (two consecutive losses of 30 Da) and two hydroxy ones (molecular weight of the TMS derivative 314 Da). Several isomers are possible: a logical structure should have a *para* –OH and two *meta* –OCH_3, so we first investigated commercially available 1,4-dihydroxy-2,6-dimethoxybenzene: compound C had retention time and spectrum coincident with 1,4-dihydroxy-2,6-dimethoxybenzene. The mean amount of 1,4-dihydroxy-2,6-dimethoxybenzene was 0.005 mg kg^{-1}, and even the maximum value reached only 0.028 mg kg^{-1} (Table 2).

The spectrum of compound D showed intense ions at *m/z* 356 (molecular ion) and *m/z* 179 (base peak) and minor ions at *m/z* 341, 267, 253 and 193 (Fig. 5B). The presence of the ion at *m/z* 267 was indicative of the structure $[(TMSO)_2C_6H_3CH_2]^+$ and the difference between the ions 356 and 267, 89 Da, was consistent with a third –OTMS. A logical structure for compound D was 3,4-Dihydroxybenzyl alcohol. This compound was synthesised, derivatized and analysed: spectrum and retention time were the same of compound D. 3,4-Dihydroxybenzyl alcohol mean amount was 0.023 mg kg^{-1}, but it reached in a sample the value of 0.31 mg kg^{-1} (Table 2).

The spectrum of compound E showed an intense molecular ion at *m/z* 384, a base peak at *m/z* 179 and the significant ion at *m/z* 267 (Fig. 5C). The difference between 384 and 267, 117 Da, could be COOTMS or –CH_2CH_2OTMS: the structure of 3,4-dihydroxyphenylacetic acid was more satisfactory than others and a commercially available standard was analysed, showing the same mass spectrum and retention time of compound E. Quantitative

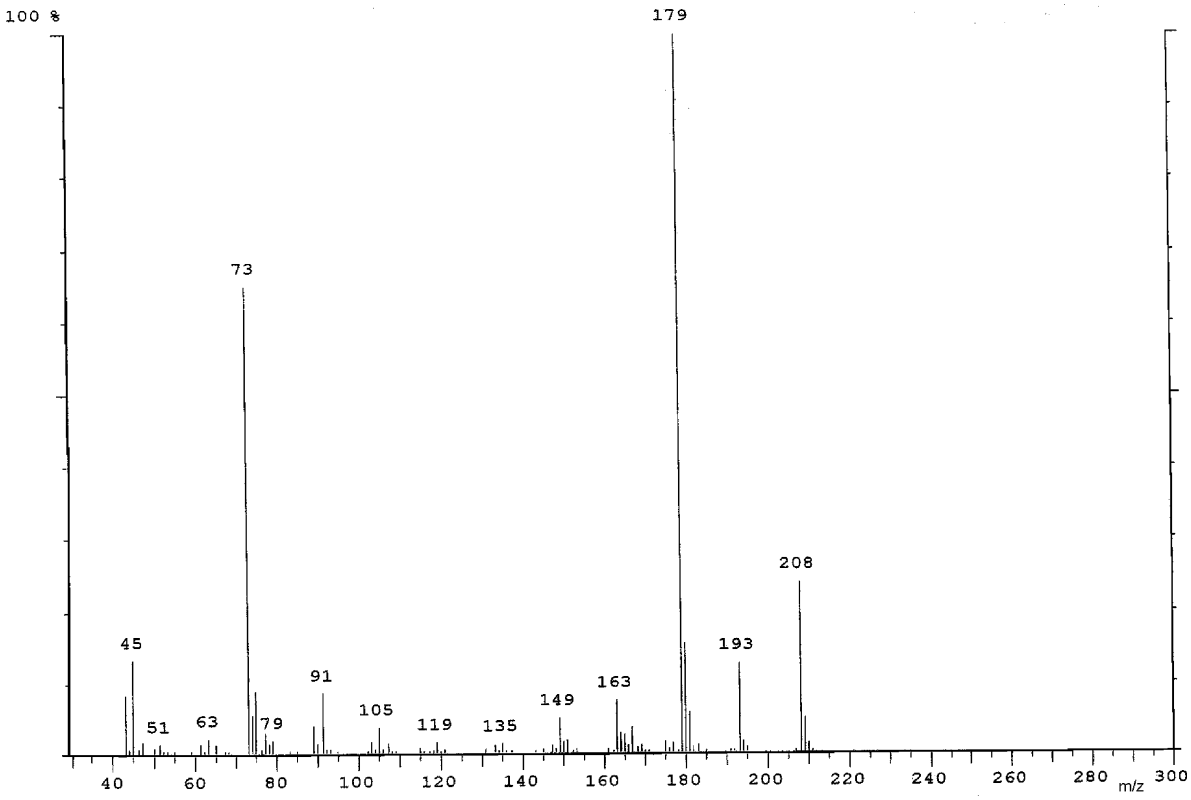

Fig. 2. Mass spectrum of 4-hydroxyphenylacetaldehyde (TMS derivative).

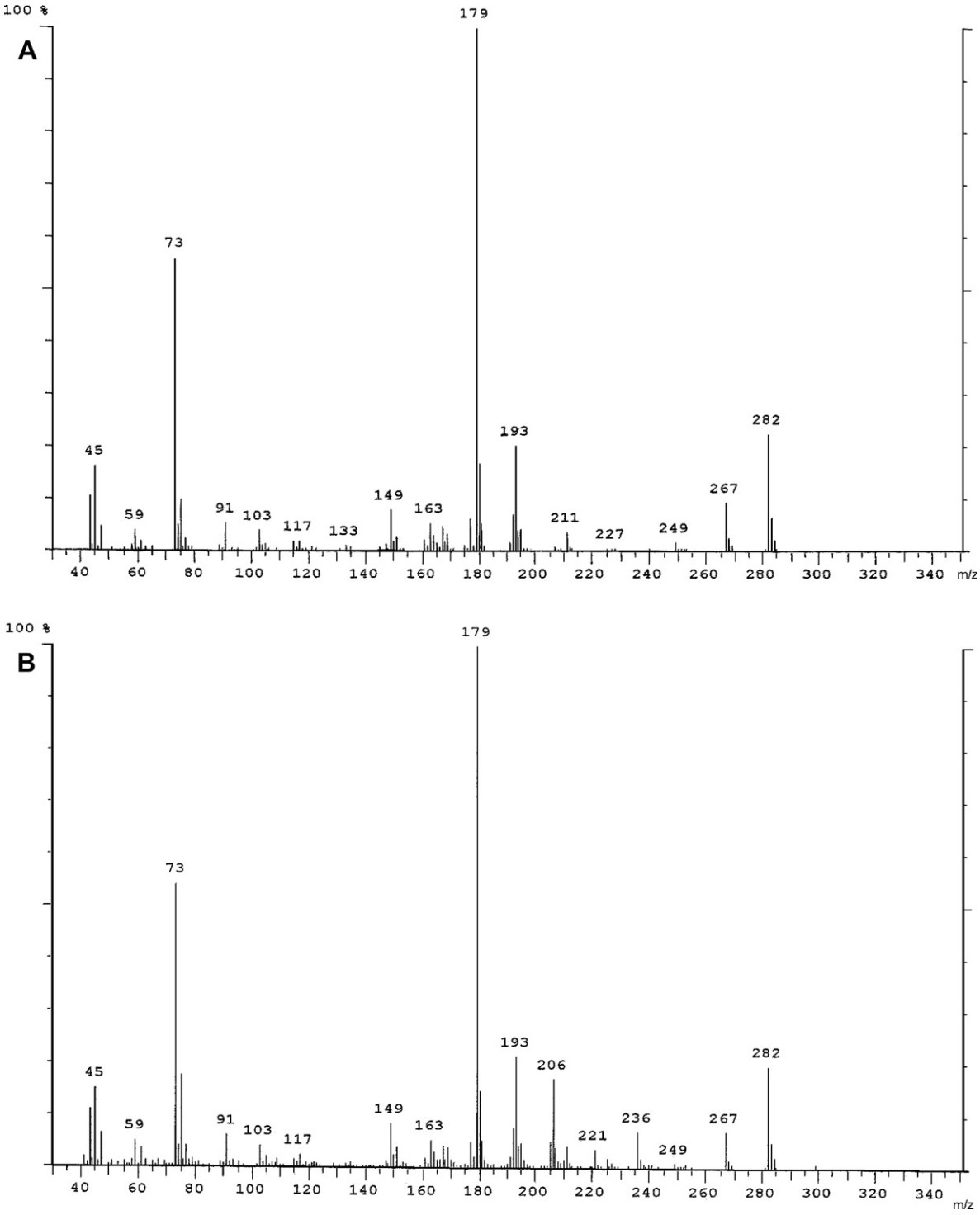

Fig. 3. (A) Mass spectrum of clean tyrosol (TMS derivative) and (B) mass spectrum of tyrosol + compound B (TMS derivatives).

data showed great differences amongst the samples, ranging from "not detected" (<0.002 mg kg^{-1}) to 1.97 mg kg^{-1}, with a mean value of 0.17 mg kg^{-1} (Table 2).

3.3. Considerations about the new compounds

The presence of 4-hydroxyphenylacetaldehyde, which is a natural oxidation product of tyrosol, was expected. Furthermore, presence of the corresponding hydroxytyrosol aldehyde has already been reported (Saitta et al., 2002). 4-Hydroxyphenylacetaldehyde has been identified in various concentrations (0.011–0.076 mg kg^{-1}) in all samples analysed.

Trans-isoeugenol, 1,4-dihydroxy-2,6-dimethoxybenzene and 3,4-dihydroxybenzyl alcohol seem to be typical components of olive oils; the first is probably synthesised by the plant following a similar biosynthetic pathway to that of cinnamic acids. All this compounds could be used as "markers" to characterise and differentiate these olive oils based on geographical origin.

3,4-Dihydroxyphenylacetic acid, which is also a naturally occurring oxidation product of hydroxytyrosol, but never found

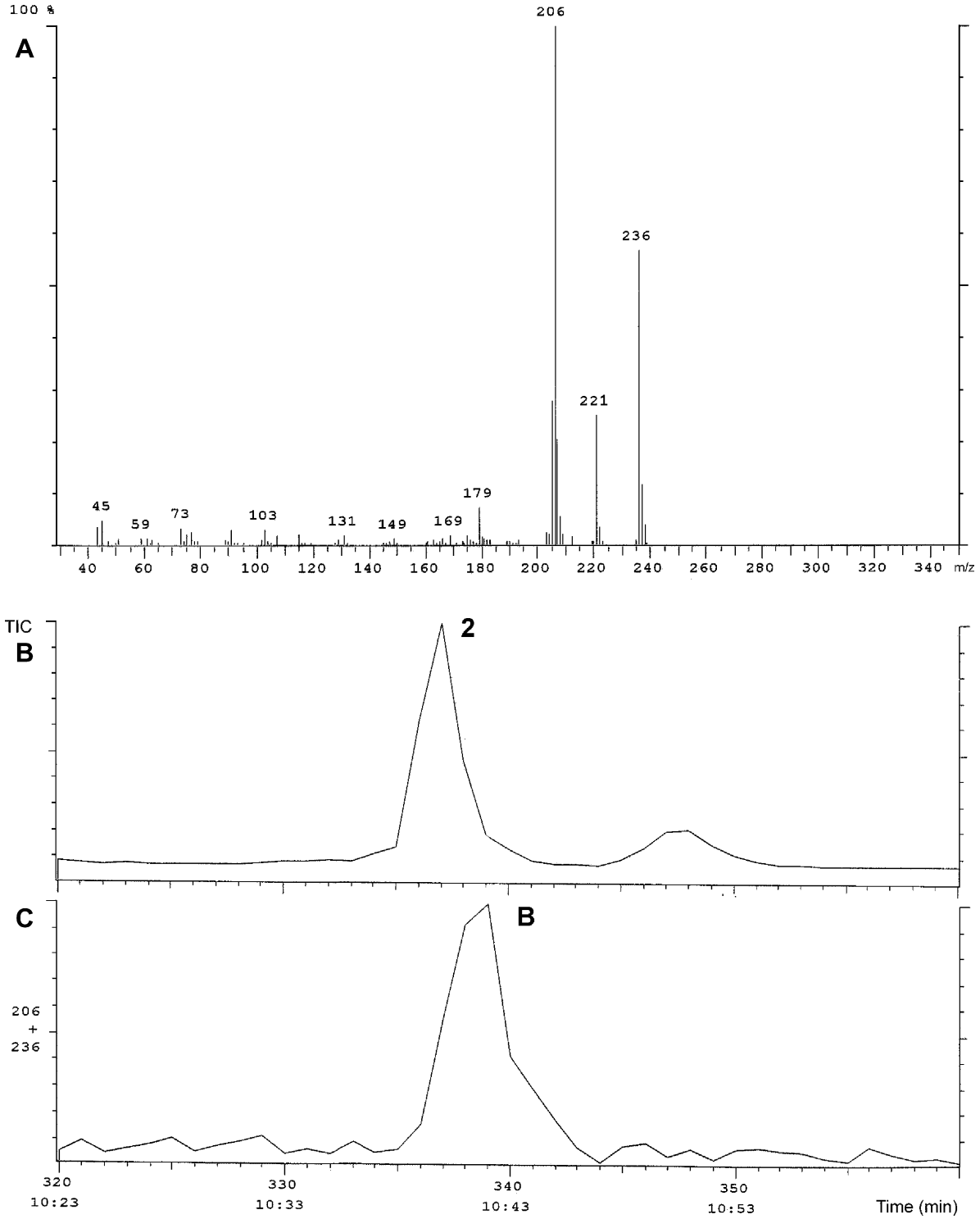

Fig. 4. (A) Mass spectrum of *trans*-isoeugenol (TMS derivative) and part of a chromatogram of a *Nocellara del Belice* olive oil sample in (B) Total Ion Current (TIC) and at (C) *m/z* 206 + 236 (Peaks identification as in Table 1).

previously in olive oils, was identified and characterised for the first time too. This compound, like other *ortho*-diphenols that are common targets in olive oil oxidation processes (Baldioli et al., 1996; Papadopoulos & Boskou, 1991), is a powerful antioxidant. It has proved to be a more powerful antioxidant than hydroxytyrosol (Fki, Allouche, & Sayadi, 2005) and has been added to olive oils to improve natural resistance to autoxidation (Blekas, Tsimidou, & Boskou, 1995). Presence of this molecule has also been reported in olive mill wastewater (Fki et al., 2005).

3,4-Dihydroxyphenylacetic acid was present in the analysed samples in variable concentrations; most samples contained less than 0.1 mg kg^{-1}, but two samples showed concentrations of 1.47 and 1.97 mg kg^{-1}, respectively. The concentration differences in the analysed samples could be explained in terms of different autoxidation stages of the various olive oils. Samples with higher concentrations of 3,4-dihydroxyphenylacetic acid (>1 mg kg^{-1}) had not yet reached the beginning stages of autoxidation and better protection against natural oxidation resulted.

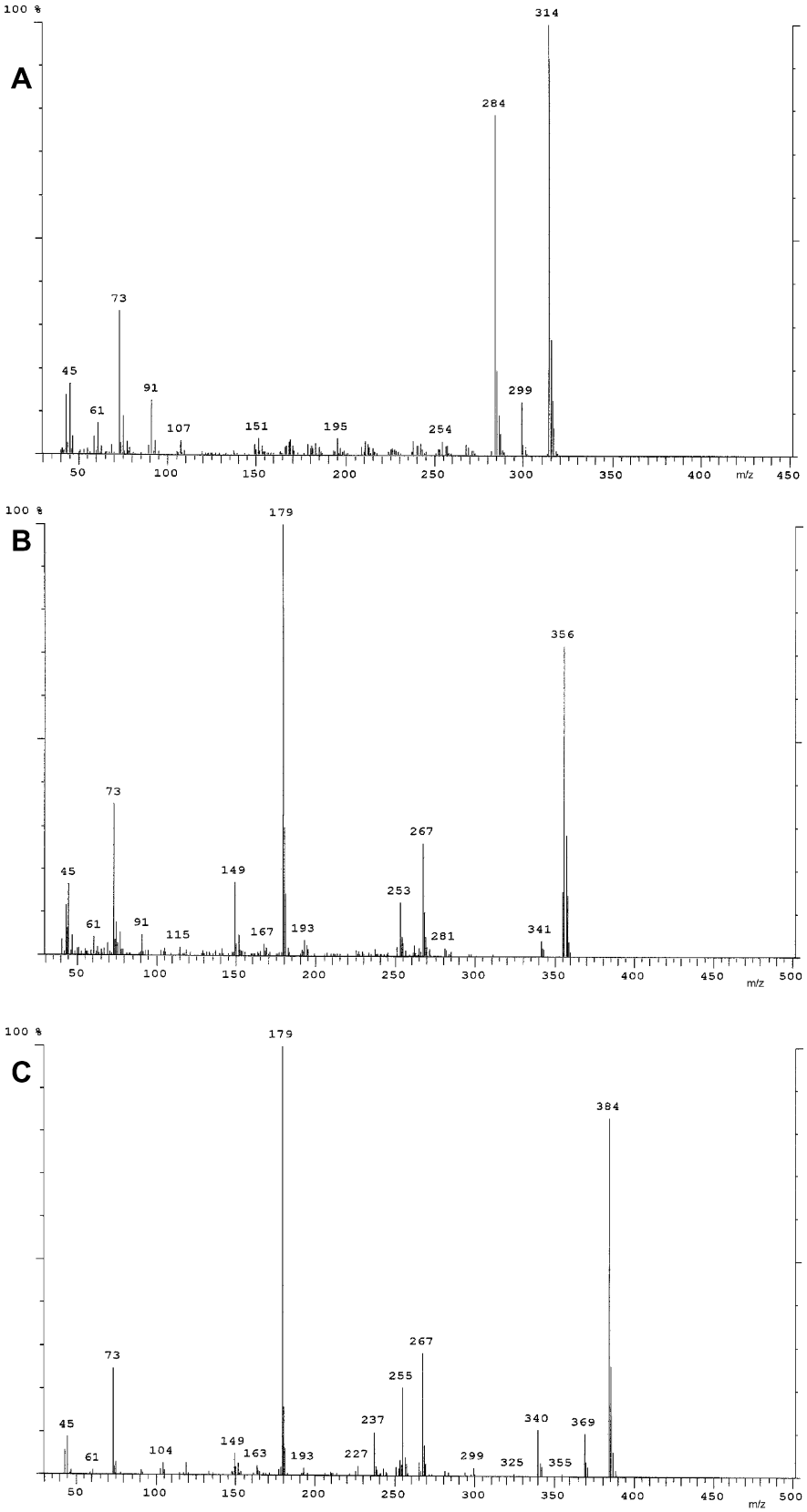

Fig. 5. (A) Mass spectrum of 1,4-dihydroxy-2,6-dimethoxybenzene (TMS derivative); (B) Mass spectrum of 3,4-dihydroxybenzyl alcohol (TMS derivative) and (C) Mass spectrum of 3,4-dihydroxyphenylacetic acid (TMS derivative).

4. Conclusion

In this paper, the phenolic fraction of 34 commercially available Sicilian oils extracted from olives of *Nocellara del Belice* showed the presence of 5 previously unidentified compounds, all at low levels; these phenols may be a peculiarity of the olive variety and useful tracers to evaluate the oil origin and quality; it is not possible to say in which manner the new compounds are produced (direct biosynthesis, oxidation of pre-existent compounds); low concentrations of 3,4-dihydroxyphenylacetic acid may show the initial autoxidation processes of olive oils.

Acknowledgements

This work was supported by "Cofin 2003 – Struttura chimica e proprieta' biologiche di componenti di oli naturali e modificati".

References

Akasbi, M., Shoeman, D. W., & Saari Csallany, A. (1993). High performance liquid chromatography of selected phenolic compounds in olive oils. *Journal of the American Oil Chemists Society, 70*, 367–370.

Amiot, M. T., Fleuriet, A., & Macheix, J. J. (1986). Importance and evolution of phenolic compounds in olive during growth and maturation. *Journal of Agricultural and Food Chemistry, 34*, 823–826.

Andrewes, P., Busch, J. L. H. C., de Joode, T., Groenewegen, A., & Alexandre, H. (2003). Sensory properties of virgin olive oil polyphenols: Identification of deacetoxy-ligstroside aglycon as a key contributor to pungency. *Journal of Agricultural and Food Chemistry, 51*, 1415–1420.

Angerosa, F., d'Alessandro, N., Konstantinou, P., & Di Giacinto, L. (1995). GC–MS evaluation of phenolic compounds in virgin olive oil. *Journal of Agricultural and Food Chemistry, 43*, 1802–1807.

Baldioli, M., Servili, M., Perretti, G., & Montedoro, G. (1996). Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *Journal of the American Oil Chemists Society, 73*, 1589–1593.

Bianco, A., Coccioli, F., Guiso, M., & Marra, N. (2001). The occurrence in olive oil of a new class of phenolic compounds: Hydroxy-isochromans. *Food Chemistry, 77*, 405–411.

Bianco, A., Mazzei, R. A., Melchioni, C., Romeo, G., Scarpati, M. L., Soriero, A., et al. (1998). Microcomponents of olive oil – III. Glucosides of 2-(3,4-dihydroxy-phenyl)-ethanol. *Food Chemistry, 63*, 461–464.

Blekas, G., Tsimidou, M., & Boskou, D. (1995). Contribution of α-tocopherol to olive oil stability. *Food Chemistry, 52*, 289–294.

Brenes, M., Garcia, A., Garcia, P. G., Rios, J. J., & Garrido, A. (1999). Phenolic compounds in Spanish olive oils. *Journal of Agricultural and Food Chemistry, 47*, 3535–3540.

Catalano, P., & Caponio, F. (1996). Machines for olive paste preparation producing quality virgin olive oil. *Fett Lipid, 98*, 408–412.

Fki, I., Allouche, N., & Sayadi, S. (2005). The use of polyphenolic extract, purified hydroxytyrosol and 3,4-dihydroxyphenyl acetic acid from olive mill wastewater for the stabilization of refined oils: A potential alternative to synthetic antioxidants. *Food Chemistry, 93*, 197–204.

Garcia, A., Brenes, M., Romero, C., Garcia, P., & Garrido, A. (2002). Study of phenolic compounds in virgin olive oils of the Picual variety. *European Food Research and Technology, 5*, 407–412.

Gerber, M. (1994). Olive oil and cancer. In M. J. Hill, A. Giacosa, & C. P. G. Caygill (Eds.), *Epidemiology of diet and cancer* (pp. 263–275). Chichester, UK: Ellis Horwood.

Gomez-Alonso, S., Salvador, M. D., & Fregapane, G. (2002). Phenolic compounds profile of Cornicabra virgin olive oil. *Journal of Agricultural and Food Chemistry, 50*, 6812–6817.

Gutierrez-Rosales, F., Rios, J. J., & 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. *Journal of Agricultural and Food Chemistry, 51*, 6021–6025.

Montedoro, G. (1972). Phenolic substances present in virgin olive oil. Note I. Identification of phenolic acids and their antioxidant power. *Science and Technology Aliment, 2*, 177–186.

Montedoro, G., & Cantarelli, C. (1969). Indagini sulle sostanze fenoliche presenti negli oli di oliva. *Rivista Italiana Sostanze Grasse, 46*, 115–124.

Montedoro, G., Servili, M., Baldioli, M., & Miniati, E. (1992a). Simple and hydrolyzable phenolics compounds in virgin olive oil, 1. Their extraction separation and quantitative and semiquantitative evaluation by HPLC. *Journal of Agricultural and Food Chemistry, 40*, 1571–1576.

Montedoro, G., Servili, M., Baldioli, M., & Miniati, E. (1992b). Simple and hydrolyzable phenolics compounds in virgin olive oil, 2. Initial characterization of the hydrolyzable fraction. *Journal of Agricultural and Food Chemistry, 40*, 1577–1580.

Owen, R. W., Mier, M., Giacosa, A., Hull, W. E., Spiegelhalder, B., & Bartsch, H. (2000). Identification of lignans as major components in the phenolic fraction of olive oil. *Clinical Chemistry, 46*, 976–988.

Papadopoulos, G., & Boskou, D. (1991). Antioxidant effect of natural phenols in olive oil. *Journal of the American Oil Chemists Society, 68*, 669–671.

Parikh, J. R., & von Doering, E. W. (1967). Sulfur trioxide in the oxidation of alcohols by dimethyl sulfoxide. *Journal of the American Oil Chemists Society, 89*, 5505–5507.

Pinelli, P., Galardi, C., Mulinacci, N., Vincieri, F. F., Cimato, A., & Romani, A. (2003). Minor polar compound and fatty acids analyses in monocultivar virgin olive oils from Tuscany. *Food Chemistry, 80*, 331–336.

Pyne, S. G., Truscott, R. J. W., Maxwell, K., Morales, M. C., Walsh, B. C., & Wynn, B. L. (1990). Model studies for insect protein sclerotization: Oxidative loss of the side chain from 4-substituted catechols. *Tetrahedron, 46*, 661–670.

Saitta, M., Lo Curto, S., Salvo, F., Di Bella, G., & Dugo, G. (2002). Gas chromatographic-tandem mass spectrometric identification of phenolic compounds in Sicilian olive oils. *Analytica Chimica Acta, 446*, 335–344.

Solinas, M., Di Giovacchino, L., & Mascolo, A. (1978). The polyphenols of olives and olive oil. Note III. Influence of temperature and kneading time and their polyphenols content. *Rivista Italiana Sostanze Grasse, 55*, 19–23.

Vazquez Roncero, A., Janer del Valle, C., & Janer del Valle, M. L. (1975). Polyphenols content and stability of olive oils. *Grasas Aceites, 26*, 14–18.