

# Analysis of extra virgin olive oils from stoned olives

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**Abstract:** This paper focuses on comparing the main chemical characteristics of 16 fresh commercial samples of extra virgin olive oil obtained from four harvest years (1999–2002) and derived from both stoned and whole fruits. The qualitative and quantitative contents of minor polar compounds (MPCs) together with other reference analytical parameters (acidity, peroxides, UV absorption values and Rancimat test) were evaluated. An investigation of the MPCs and oil composition obtained from only stoned olives was also carried out. The acidity values of the oils from stoned fruits were always similar to or lower than those of the corresponding oils from whole fruits. For almost all the samples from stoned olives a better resistance to oxidation was revealed in comparison with the corresponding traditional oils. Five pairs obtained from the 2000 and 2001 harvests showed higher concentrations of both MPCs and hydroxytyrosol derivatives in the oils from stoned fruits, in agreement with their higher Rancimat values. Overall, our findings with regard to acidity values, % hydrolysis, the Rancimat test and the qualitative and quantitative distribution of MPCs suggest a higher antioxidant capacity of the oils from stoned olives.

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**Keywords:** extra virgin olive oil; stoned olives; minor polar compounds; HPLC/DAD; Rancimat

## INTRODUCTION

The nature and composition of olive fruit together with the applied milling procedures, which are exclusively physical methods, are the reason for the substantial differences between extra virgin olive oils and other vegetable oils. Extra virgin olive oil is becoming more relevant in the diet of several countries owing to its beneficial effects on human health. Epidemiological studies involving Mediterranean populations have revealed a positive correlation between olive oil intake and reduced risk of cardiovascular diseases.<sup>1–3</sup> Moreover, the data available up till now also allow the establishment of a positive correlation between olive oil intake and decreased risk of tumour development.<sup>4,5</sup> Some of these effects are presumably associated with the content of phenolic compounds and the high amount of oleic acid as well as tocopherols present in extra virgin olive oils.<sup>5–8</sup>

Currently, both traditional and more modern milling processes are based on whole fruit treatment. The idea of obtaining extra virgin olive oil from only the pulp was already proposed in 1794 in the treatise 'Degli ulivi e delle ulive e della maniera di cavar l'olio' by Giovanni Presta. The author described the possibility 'to extract the oil from stoned olives', stating that the product was preferred by consumers of the time. Nowadays, some authors suggest the possibility of industrially manufacturing extra virgin

olive oil from stoned olives,<sup>9,10</sup> demonstrating that the removal of the pit reduces the oil yield by only 1.5%.<sup>11</sup> Moreover, from studies carried out on pits from different cultivars, it has been shown that this matrix contains complex glycosidic structures,<sup>11,12</sup> among which nüzhenide is the most abundant.<sup>13</sup> It should be noted that these secoiridoidic derivatives do not contain hydroxytyrosol, but rather one or more tyrosolic nuclear moieties, and thus do not substantially contribute to the antioxidant potential of the oil.

Recently, commercial extra virgin oils from stoned olives have been found on the market, because the applied technology makes it possible to remove the stone completely without the loss of any pulp. Both traditional and continuous extraction processes extract the oil by breaking down the fruit through pressing and crushing. The physical aspect of the paste is normally granular, because, together with the pulp, pieces of pit are also present and play a draining role in the paste. The extraction process for extra virgin olive oil from stoned olives is therefore radically different from the usual processes, since the paste is smoother and more homogeneous compared with that obtained from whole olives.<sup>14,15</sup>

The enzymes LPO (lipooxygenase) and POD (peroxidase) are found in both the pit and pulp of olives

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at variable concentrations, depending on the degree of ripeness, and in some cases POD has been reported to be more abundant in the stone.<sup>16</sup> Other authors have evidenced more intense LPO activity during malaxation with pieces of pit;<sup>10,17</sup> therefore the removal of the pit could lead to a lesser quantity of these enzymes in the paste, thus reducing oxidative processes. Recently, contrasting evidence was obtained in a study on fruits from different Italian cultivars harvested during a single year. The authors showed a lower content of LPO in the stone with respect to the pulp, and no differences were found in the paste obtained by milling whole fruits or stoned olives.<sup>18</sup>

In the present work, several pairs of commercial extra virgin olive oils obtained from four harvest years (1999–2002) and produced by a traditional process and an innovative milling process on stoned fruits were analysed. The qualitative and quantitative contents of minor polar compounds (MPCs) together with other quality analytical parameters (acidity, UV absorption values and peroxides) were evaluated. Moreover, oxidative stability was measured by the Rancimat test for the oils obtained from whole fruits and stoned olives. Finally, an investigation of the MPCs and oil composition obtained from olive pits was also carried out.

## MATERIALS AND METHODS

### Oil samples

Eight pairs of commercial extra virgin olive oils obtained from whole fruits and stoned olives were analysed. Each pair was obtained from the same batch of olives (ranging from 300 to 1000 t) and produced by industrial milling. Fourteen oils of cvs Coratina (C) and Peranzana (P) from Italy and two oils from Spain (S) were obtained by traditional milling (T) or from stoned olives (S). The Italian samples denoted 99, 00 and 02 were harvested in the period 15 November–15 December of the years 1999, 2000 and 2002 respectively. The Spanish samples were harvested in the last 2 weeks of January 2002 and are denoted ST 01 and SS 01.

The oil samples from whole fruits were extracted by a continuous system with the following process phases: washing, crushing (hammer crusher), malaxation, centrifugation and separation. The new process adopted for the production of oils from stoned olives is different from the standard one, because the crushing machine is replaced by a depitting machine (Alfa Laval X 32, 2–25 t h<sup>-1</sup>). Both milling systems were from Alfa Laval (Firenze, Italy) and the following conditions were applied: kneading temperature 28–30 °C kneading time 45–60 min and process temperature 30–32 °C.<sup>13,14</sup>

### Chemicals

Oleuropein, tyrosol (Tyr) and luteolin (Lut) were purchased from Extrasynthese (Lyon, France) Hydroxytyrosol (OH-Tyr) was purchased from Cayman

Chemical (SPI-BIO-Europe, Montigny le Bretonneux, France). All other reagent and solvents were of analytical grade.

### Sample preparation

#### MPC fraction

Time intervals between production of the oils and analysis ranged from 1 to 4 weeks. In particular, the panel test and all the common chemical tests were performed within 8–10 days of milling. All the oils were stored at –20 °C before the analyses. The determination of MPCs was performed 1 month after milling.

A volume of 25 ml of each oil sample was extracted with 75 ml of EtOH/H<sub>2</sub>O (70:30 v/v), using water acidified with formic acid (pH 2.5), and stirred for 20 min. Defatting with *n*-hexane (3 × 25 ml) was performed to completely remove the lipid fraction. The hydro-alcoholic extract of each sample was concentrated under reduced pressure to dryness, rinsed with 2 ml of extraction solvent and then analysed by high-performance liquid chromatography/diode array detection (HPLC/DAD) and HPLC/mass spectrometry (HPLC/MS).

#### Coratina pit

A sample of Coratina pits (about 500 g) collected in November 2000 was used to evaluate the oil content and its composition together with the MPC fraction. The pits were crushed in a laboratory mill (Reactor 15 320, Foss Electric, Padua, Italy) with water (2:1 w/w) to yield a homogeneous sample. Subsequently 1 l of EtOH/HCOOH (98:2 v/v) and 1.5 l of *n*-hexane were added and mixed. The upper phase (EtOH/*n*-hexane) was removed and the extraction was repeated with 2 × 700 ml of *n*-hexane. All the upper phases were collected, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and distilled to yield an oil sample of 3.57% of the pit weight. The oil was analysed as described below, while the lower aqueous phase was filtered and the hydro-alcoholic solution was directly analysed by HPLC/DAD for the MPC determination.

#### Hydrolysis conditions of MPC fraction

To 300 µl of the hydro-alcoholic solution containing the MPCs from Coratina pit was added 300 µl of 1 mol l<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>, and the mixture was maintained at 80 °C for 2 h in an oven. The sample was then diluted with 400 µl of ethanol to obtain a clear solution directly analysable by HPLC/DAD. These experimental conditions were adequate to avoid degradation of both Tyr and OH-Tyr, as verified by carrying out the same test with pure standards.

#### HPLC/DAD/MS analyses of MPCs

The analysis of MPCs was performed using an HP 1100 liquid chromatograph equipped with diode array and mass spectrometric detectors and an MSD atmosphere pressure ionization (API)–electrospray interface (all from Hewlett-Packard, Palo Alto,

CA, USA). The column used was a LiChrosorb RP18 (5  $\mu$ m)250 mm  $\times$  4.6 mm id column (Merck, Darmstadt, Germany) maintained at 26 °C and equipped with a 10 mm pre-column of the same phase. The eluents were H<sub>2</sub>O (pH 3.2 with HCOOH) and CH<sub>3</sub>CN. A multi-step linear solvent gradient from 100% H<sub>2</sub>O to 100% CH<sub>3</sub>CN was used over a 106 min period at a flow rate of 1 ml min<sup>-1</sup> as previously reported.<sup>19,20</sup> A Luna C18 (5  $\mu$ m)250 mm  $\times$  4.6 mm column (Phenomenex, Torrance, CA, USA) was used to perform the flavonoid determination. This stationary phase guarantees higher efficiency and therefore a more accurate evaluation of these compounds, which are always present in small amounts. The following multi-step linear gradient was applied: from 100 to 85% A in 5 min; to 70% A in 10 min and a plateau of 5 min; to 65% A in 5 min and a plateau of 5 min; to 55% A in 7 min and a plateau of 5 min; to 100% B in 5 min and a final plateau of 3 min. The total time of analysis was 50 min, the equilibration time 20 min and the oven temperature 26 °C. The solvents were (A) H<sub>2</sub>O/0.1% HCOOH and (B) CH<sub>3</sub>CN at a flow rate of 1 ml min<sup>-1</sup>.

The quantitative evaluation of individual phenols was performed using four-point regression curves ( $r^2 \geq 0.999$ ) and authentic standards. Tyr and OH-Tyr were evaluated at 280 nm using tyrosol as standard. The dialdehydic form of elenolic acid (EA) linked to OH-Tyr (3,4-DHPEA-EDA) and the other secoiridoids (Other Sec) were evaluated at 280 nm using oleuropein as standard. EA was evaluated at 240 nm using oleuropein as standard. For 3,4-DHPEA-EDA and EA the following correction factors of the molecular weight (MW) were respectively applied: 320/540 and 242/540 (540 = MW of oleuropein). Luteolin and apigenin were evaluated at 350 nm using luteolin as standard.

### Other analyses

Acidity value, peroxide index, UV absorption, fatty acid composition, sterols, triglycerides and  $\Delta$ ECN42 (defined as the difference between the content of triacylglycerol with an equivalent carbon number of 42 obtained by HPLC analysis and the theoretical content calculated on the basis of fatty acid composition by GC analysis) were determined by the official analytical methods described in regulation EEC/2568/91 of the European Union Commission.

Oxidative stability was determined by the Rancimat test (Metrohm Instruments model 679, Herisau, Switzerland) on 5 g of oil at 110 °C and 20 l h<sup>-1</sup> airflow, and the curves were registered at 1 cm h<sup>-1</sup> as previously described.<sup>21,22</sup>

Sensory analyses by panel test were carried out by the official methods described in regulation EEC/2568/91.

## RESULTS AND DISCUSSION

Most of the oils were collected in the years 2000–2002, since in 1999 only a pair of oils from cv Coratina

were available, because the process for obtaining commercial oil from stoned olives was still in a developmental stage. To carry out analysis and comparison of the quality of the eight pairs of oils, efforts were mainly directed towards the evaluation of MPCs together with other chemical and organoleptic parameters.

It is well known that extra virgin olive oil is the only vegetal oil for human consumption that naturally contains appreciable amounts of MPCs, largely responsible for oil stability with respect to self- and thermo-oxidation. According to the findings of other studies, this fraction is complex and comprises simple phenols, eg Tyr and OH-Tyr, cinnamic acids,<sup>23,24</sup> secoiridoidic derivatives, flavonoids in trace amounts<sup>20,25</sup> and lignans, mainly found in Spanish oils<sup>26–28</sup> but also in Italian extra virgin olive oil.<sup>6</sup>

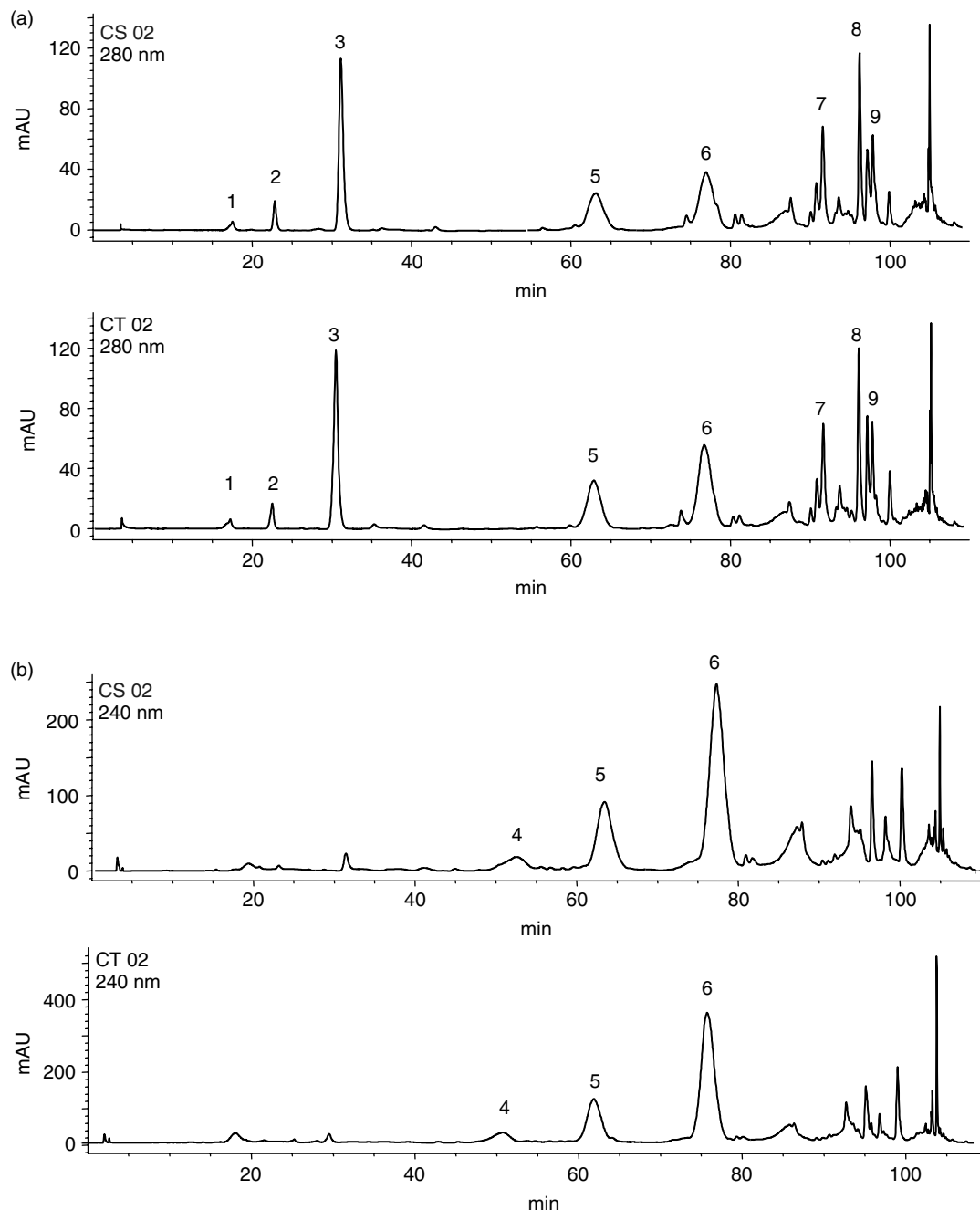
The HPLC profiles of the MPC fractions were similar and often overlapped (Fig 1) for almost all the pairs of oil samples (S and T), while the quantitative amounts presented notable variations. The histogram in Fig 2 shows a comparison among four different chemical classes within the MPCs, expressed as %  $\Sigma$  of each group. It appears evident that the secoiridoidic fraction usually varies around 80% or more. Observing the values in Table 1, for several oils, 3,4-dihydroxyphenylethanol elenolic dialdehydic acid (3,4-DHPEA-EDA), having an *o*-diphenolic group, is often one of the main compounds within this class, as previously highlighted.<sup>20,25</sup>

For each oil pair, differences were evidenced in the % MPC hydrolysis, defined as [(Tyr + OH-Tyr)/total phenols]  $\times$  100. For almost all the oils, higher values of this parameter were evidenced for samples obtained by milling whole fruits. In aged extra virgin olive oils an increase in this parameter was previously verified,<sup>29,30</sup> so the quality of fresh oil can also be correlated with lower values of % MPC hydrolysis.

With regard to the flavonoidic fraction, the total amounts ranged between 0.8 and 7 mg l<sup>-1</sup> for all the samples, confirming a very low content of these molecules in the oils, as previously described.<sup>20,25</sup> For each pair of oils, no differences were evidenced regarding the flavonoidic fraction.

The main results of MPC analyses were obtained at 280 nm and are summarised in Table 1. In these conditions, EA and its analogues were not evaluated. The highest phenolic contents were encountered, with a few exceptions, in the oils from stoned olives. Particularly high levels of MPCs were found in Coratina and Peranzana samples from 2001, while the same pair of oils obtained in 2002 exhibited lower levels. It should be noted that the 2002 harvest was characterised by olives which were partially damaged by *Bactrocerae oleae*, and this may have negatively influenced the total MPC amounts.

Table 2 summarises the results for the most common analytical parameters evaluated in the oils: free acidity, peroxide number, UV absorbance and the time

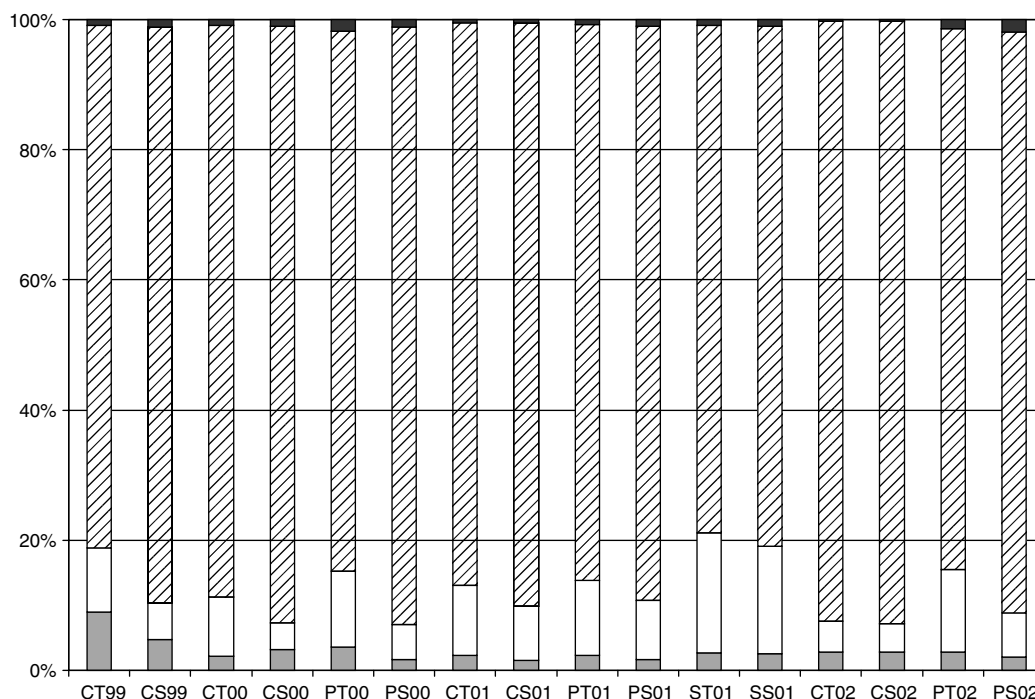


**Figure 1.** Comparison of chromatographic profiles at (a) 280 nm and (b) 240 nm of MPC fraction from CS 02 and CT 02: 1, OH-Tyr; 2, Tyr; 3, ID (internal standard); 4, EA; 5, 3,4-DHPEA-EDA; 6, 7, 8 and 9, secoiridoidic derivatives.

of oxidation induction evaluated by the Rancimat test. Almost all the samples showed an acidity below 0.5%, and the values for the oils from stoned fruits were similar to or lower those for the corresponding oils from whole fruits (CS 99, CS 01, PS 00, CS 02). The peroxides were almost identical for each pair of oils.

For the UV absorbance measurements, all data were within the limits set by official regulations, and no differences were evidenced among traditional oils and stoned oils. These chemical parameters, together with the panel test, showed no differences due to the presence or absence of the stone, in agreement with results recently published by Patumi *et al*<sup>18</sup> on several pairs of oils obtained from a two-phase decanter.

The rancimat test directly provides the induction time for oxidation of the oil at the selected temperature, giving a comparative measurement of the resistance of the sample to forced oxidation. For almost all the oils from stoned olives, higher induction times were obtained compared with the whole fruit oils, as shown in Table 1. According to other authors,<sup>22,31</sup> the Rancimat results can be correlated with the amount of MPCs. In particular, the samples from the 2000 and 2001 harvests showed higher concentrations of both 3,4-DHPEA-EDA and total MPCs in the oils from stoned fruits, in agreement with their higher induction times. The only exception was the CS 02 and CT 02 pair, which showed a



**Figure 2.** MPC content expressed as %  $\Sigma$  of four different chemical classes: Tyr + OH-Tyr (grey); flavonoidic compounds (black); EA + EA derivatives (white); secoiridoidic molecules (hatching).

**Table 1.** Amounts of phenolic compounds ( $\text{mg l}^{-1}$ ) in oils as determined by HPLC/DAD analysis at 280 nm

Oil	OH-Tyr	Tyr	3,4-DHPEA-EDA <sup>a</sup>	Secoiridoidic compounds <sup>a,b</sup>	Total
CS 99	1.3 $\pm$ 0.1	5.8 $\pm$ 0.1	1.9 $\pm$ 0.1	129.7 $\pm$ 2.7	138.7
CT 99	12.5 $\pm$ 0.7	17.9 $\pm$ 0.2	22.4 $\pm$ 0.4	248.6 $\pm$ 1.0	301.4
CS 00	10.3 $\pm$ 0.4	8.5 $\pm$ 0.2	120.0 $\pm$ 1.9	416.4 $\pm$ 1.1	555.1
CT 00	4.4 $\pm$ 0.3	6.3 $\pm$ 0.4	52.4 $\pm$ 0.9	379.0 $\pm$ 2.2	442.0
CS 01	8.9 $\pm$ 0.3	9.5 $\pm$ 0.2	171.9 $\pm$ 1.9	865.6 $\pm$ 12.2	1055.8
CT 01	5.5 $\pm$ 0.3	11.6 $\pm$ 0.3	97.6 $\pm$ 1.6	546.9 $\pm$ 2.8	661.5
CS 02	2.7 $\pm$ 0.1	6.1 $\pm$ 0.4	62.5 $\pm$ 0.8	231.4 $\pm$ 2.6	302.6
CT 02	3.3 $\pm$ 0.2	6.2 $\pm$ 0.4	69.2 $\pm$ 0.6	238.7 $\pm$ 1.8	317.3
PS 00	5.3 $\pm$ 0.3	4.4 $\pm$ 0.4	169.8 $\pm$ 1.3	359.8 $\pm$ 2.6	539.3
PT 00	8.3 $\pm$ 0.3	6.0 $\pm$ 0.2	114.4 $\pm$ 1.1	209.8 $\pm$ 2.2	338.4
PS 01	6.2 $\pm$ 0.1	4.8 $\pm$ 0.4	123.1 $\pm$ 0.4	473.6 $\pm$ 2.3	607.6
PT 01	6.0 $\pm$ 0.2	6.6 $\pm$ 0.5	111.4 $\pm$ 0.3	355.9 $\pm$ 0.5	479.8
PS 02	2.2 $\pm$ 0.1	2.8 $\pm$ 0.2	91.5 $\pm$ 0.9	128.6 $\pm$ 1.5	225.1
PT 02	3.6 $\pm$ 0.2	4.5 $\pm$ 0.4	86.7 $\pm$ 0.8	154.8 $\pm$ 2.1	249.5
SS 01	10.3 $\pm$ 0.1	6.4 $\pm$ 0.1	128.4 $\pm$ 0.5	394.1 $\pm$ 2.2	539.1
ST 01	8.7 $\pm$ 0.4	7.4 $\pm$ 0.5	115.1 $\pm$ 0.5	347.2 $\pm$ 2.3	478.3

Values are mean  $\pm$  standard deviation of three determinations.

<sup>a</sup> Applying the *t*-test at 95% probability level, the values for each pair of oils are statistically different, with the sole exception of CS 02 and CT 02.

<sup>b</sup> These secoiridoidic molecules include the OH-Tyr and Tyr moieties.

similar MPC content but a different resistance to forced oxidation.

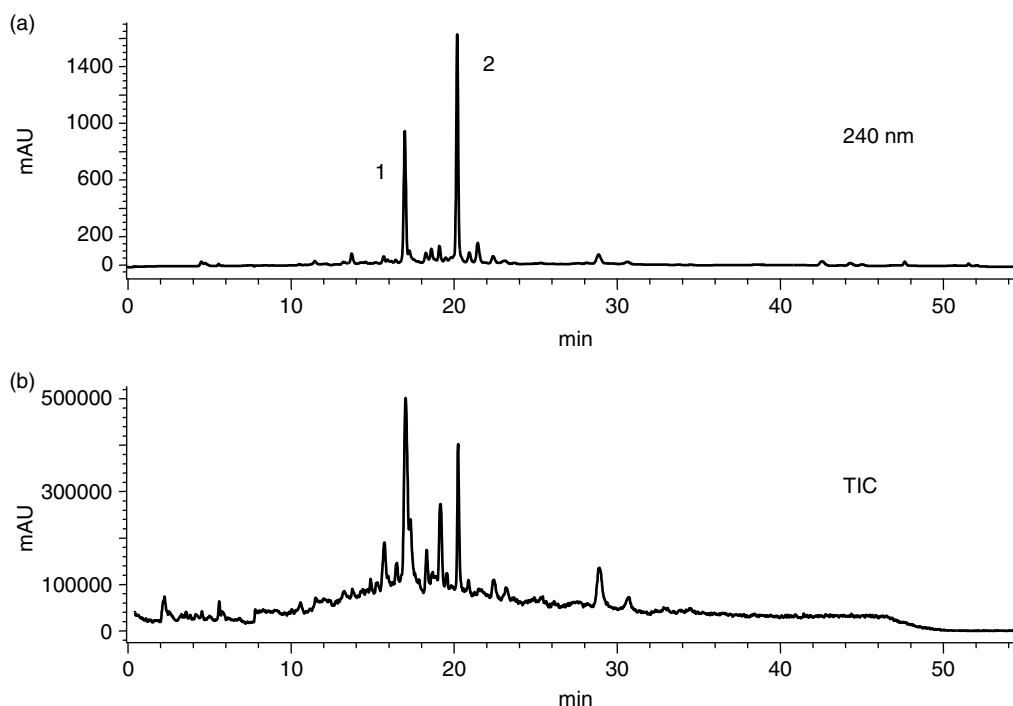
Together with the analysis of extra virgin olive oils, a study on the pit composition was also carried out. The oil obtained from a sample of Coratina pits harvested in 2000 was analysed, measuring the same parameters considered for the corresponding pair of extra virgin oils. Observing the main findings relating to fatty acid composition, triglyceride content and sterolic content (summarised in Table 3), this oil can be defined as a typical seed oil. In fact, higher values of linoleic acid (L), trilinolein (LLL),

$\Delta\text{ECN}42$ ,  $\Delta 7$ -stigmastenol and total sterols were observed with respect to the corresponding CS 00 and CT 00 extra virgin olive oils. The decrease in oil yield on going from whole fruits to stoned olives, calculated after industrial milling, ranged between 1 and 1.5%.<sup>14</sup> A previous study indicated a pit oil yield, obtained by a laboratory milling process, near 1.5%.<sup>11</sup> Our results confirm this value. The reduction in oil yield for the samples from stoned fruits seems to be related to the absence of the draining effect due to pieces of pit during malaxation.<sup>11,14</sup>

**Table 2.** Summary of results from several common analyses carried out on oil samples

Oil	Acidity <sup>a</sup> (% oleic acid)	Peroxides <sup>b</sup> (meq O <sub>2</sub> kg <sup>-1</sup> )	K <sub>232</sub> <sup>c</sup>	K <sub>270</sub> <sup>d</sup>	ΔK <sup>e</sup>	Panel test score	Rancimat test (h)
CS 99	0.23	10.7	2.02	0.131	-0.001	7.5	—
CT 99	0.43	10.2	1.85	0.113	-0.001	7.0	—
CS 00	0.16	5.18	1.69	0.124	-0.004	7.5	28.5
CT 00	0.19	6.71	1.76	0.116	-0.003	7.5	21.6
CS 01	0.20	7.6	2.09	0.16	-0.005	7.5	28.8
CT 01	0.42	9.5	2.10	0.17	-0.002	6.5	19.3
CS 02	0.44	6.8	1.67	0.13	-0.001	7	23.4
CT 02	0.62	9.1	1.68	0.2	-0.001	6.5	17.9
PS 00	0.36	8.38	1.64	0.117	-0.001	7.5	22.7
PT 00	0.54	7.90	1.81	0.122	-0.003	7.5	18.7
PS 01	0.42	10.0	2.14	0.15	-0.003	7.0	22.7
PT 01	0.48	10.9	2.12	0.15	-0.002	7.0	18.9
PS 02	0.19	6.0	1.85	0.11	-0.00	7.5	22.9
PT 02	0.25	6.7	1.72	0.12	-0.001	7.5	23.0
SS 01	0.43	7.1	1.66	0.12	-0.006	7.0	41.2
ST 01	0.42	8.9	1.73	0.14	-0.006	7.0	35.0

Values are mean of three determinations. The following relative standard deviations (RSD) were obtained: <sup>a</sup> RSD < 3%, <sup>b</sup> RSD < 5%, <sup>c</sup> RSD < 4.8%, <sup>d</sup> RSD < 6%, <sup>e</sup> RSD < 30%.

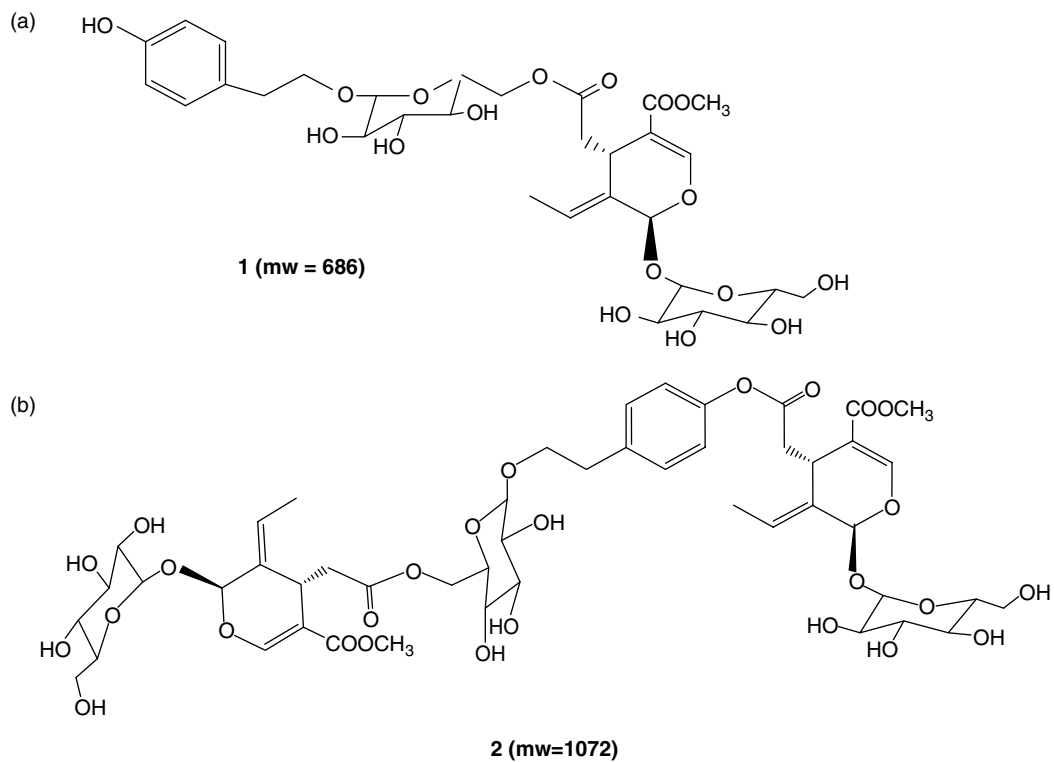


**Figure 3.** MPCs from Coratina pit harvested in 2000: (a) chromatographic profile at 240 nm; (b) total ion current (TIC) profile in negative ionisation mode at Fragmentor 150V.

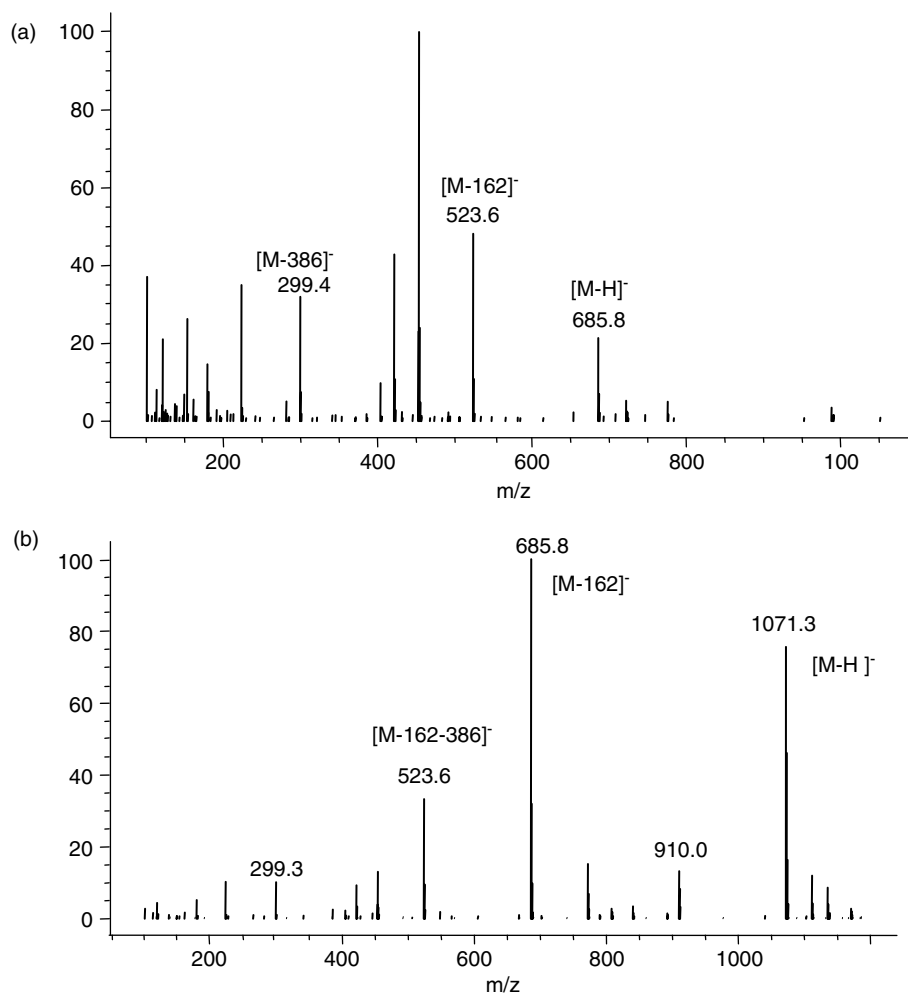
Moreover, as evidenced in the HPLC/DAD profile at 240 nm (Fig 3), the MPCs from Coratina pits showed a simple chromatographic pattern with the presence of only two main peaks. After HPLC/DAD/MS investigations they were identified with the structures shown in Fig 4, as nüzhenide according to Servili *et al*<sup>13</sup> and as a secoiridoid triglycoside previously reported in Spanish cultivars.<sup>26–28</sup> The electrospray ionisation mass spectra in negative ionisation mode (Fig 5) show for both these compounds the quasi-molecular ions and other diagnostic fragments relating to the loss of the glucosidic group

alone, ie  $[M - 162]^-$ , or with the secoiridoid group, ie  $[M - 386]^-$ . The spectra obtained in positive mode allowed confirmation of the molecular weights of these glycosides. Finally, it was verified by acidic hydrolysis that none of these molecules contained the OH-Tyr group, but only Tyr moieties. It is known that Tyr shows a negligible contribution to the antioxidant properties of the oil when compared with OH-Tyr activity,<sup>32,33</sup> so the MPCs from the pit do not improve the antioxidant capacity of extra virgin oils.

The main findings from this research have shown interesting differences in acidity value, % hydrolysis,



**Figure 4.** Chemical structure of (a) nüzhenide and (b) secoiridoid **2** from pit extract.



**Figure 5.** Mass spectra of (a) nüzhenide and (b) secoiridoid **2** obtained in negative ionisation mode at Fragmentor 150 V.

**Table 3.** Results from main chemical analyses applied to three oils obtained from same batch of olives (Coratina, 2000 harvest): whole fruit (CT), stoned fruit (CS) and pit (C)

Chemical parameter	CS	CT	C
Oleic acid <sup>a</sup>	77.8	77.4	69.9
Linoleic acid <sup>a</sup>	7.5	7.5	15.0
Linolenic acid <sup>a</sup>	0.7	0.7	0.4
LLL <sup>b</sup>	0.05	0.08	1.01
ECN42	0.38	0.38	1.27
ΔECN42	0.01	0.03	0.63
Δ7-Stigmastenol <sup>a</sup> (%)	0.1	0.2	0.8
Campesterol <sup>a</sup> (%)	3.3	3.4	4.3
∑ β-Sitosterol <sup>a,c</sup> (%)	94.6	94.2	92.5
Total sterols (mg kg <sup>-1</sup> )	1099	1043	4939

Values are mean of three determinations.

<sup>a</sup> RSD < 1.5%.

<sup>b</sup> RSD < 15%.

<sup>c</sup> Corresponding to β-sitosterol + cleroosterol + sitostanol + Δ5-avenasterol + Δ5-24-avenasterol.

Rancimat test and MPC content between these two types of extra virgin olive oil. These results, though related to only a limited numbers of fresh oil samples, suggest a better quality of these new commercial oils with respect to antioxidant capacity, which is closely correlated with the shelf life of the product. In addition, the use of stoned fruits in the milling process may open up new perspectives, especially if it is possible to easily recover the whole pit, which can become a valuable by-product.

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