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Phenolic compounds of virgin olive oil: influence of paste preparation techniques

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Abstract

An experimental investigation was carried out on olive oils of the *Ogliarola Salentina* and *Coratina* cultivars to assess the influence of the two different olive grinding techniques and kneading process on the quality of the oils. The experimental data obtained showed that resistance to oxidation, total phenols and phenolic compounds analysed for HPLC were higher in the *Coratina* oils than in the *Ogliarola* oils. The use of hammer-crushers plus kneader rather than stone mills plus kneader always produced significant increases in the total phenols. Resistance to oxidation was assessed by the Rancimat method and showed a significant correlation with the amounts of total phenols and of an unidentified substance (peak I) which was conspicuously present amongst the substances evaluated for HPLC. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Total phenols; Resistance to oxidation; HPLC; Phenolic compounds

1. Introduction

Triglyceride auto-oxidation reactions constitute major changes occurring in edible vegetable oils. The subsequent alterations make the oil unsuitable for direct human consumption. Virgin olive oil contains a number of substances which influence its shelf-life. Amongst them, phenolic compounds are the ones that mainly determine a greater resistance to auto-oxidation (Vázquez Roncero, Janer del Valle, & Janer del Valle, 1975; Gutiérrez González-Quijano, Janer del Valle, Janer del Valle, Gutiérrez Rosales, & Vázquez Roncero, 1977; Perrin, 1992; Tsimidou, Papadopoulos, & Boskou, 1992; Baldioli, Servili, Perretti, & Montedoro, 1996). The interest surrounding this class of compounds is due to the fact that they are considered responsible for conferring specific organoleptic properties to the oil (Gutiérrez González-Quijano et al., 1977; Vázquez Roncero, 1978). Phenolic compounds may confer a marked 'bitter to pungent' taste or a 'sweet' taste typical of some virgin olive oils (Montedoro & Servili, 1991). This is particularly true with oils obtained from drupes of the *Coratina* cultivar, which are typically 'bitter and pungent', or from olives of the *Ogliarola Salentina* cultivar, which produce characteristically 'sweet' tasting oils.

The aim of this work was to perform a comparative examination regarding the qualitative and quantitative evaluation of phenolic compounds by HPLC in oils obtained from both these cultivars as well as to assess how the different olive grinding techniques and the kneading process affected the quality of the oil. The possible consequences that phenolic compounds could have on the resistance to oxidation and on the organoleptic properties of the oils were also taken into account.

2. Materials and methods

2.1. Sample preparation

The samples consisted of hand-picked olives of the *Ogliarola Salentina* and *Coratina* cultivars from Apulia. The olives were picked at an optimal stage of ripeness during the 1994/1995, 1995/1996, and 1996/1997 olive-oil years. Ten different batches were taken for both cultivars and each batch was representative of a broad olive-growing area. After homogenisation, washing, and

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leaf-removal, each batch was divided into two portions, namely one of about 2.5 q which was milled for 20 min with a 4-stone mill at an industrial oil-processing plant and another of about 1.5 q which was hammer-crushed in a pilot plant (Catalano & Caponio, 1996). The olive pastes thus obtained were divided into two fractions of which only one was kneaded for 20 min at 20–25°C. Homogeneous samples of paste of at least 5 kg were then collected from each fraction and the oil was extracted by means of a laboratory basket centrifuge. Hence, for each batch of olives, four different oils were extracted, namely one from the milled paste, one from the milled and kneaded paste, one from the crushed paste, and one from the crushed and kneaded paste. All the oil samples were filtered through cotton before being analysed.

2.2. Reagent and standard

The following standards were used: (*p*-hydroxyphenyl)ethanol, gallic, protocatechuic, *p*-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, sinapic, *o*-coumaric, cinnamic acids and apigenin from Fluka (Buchs, Switzerland); 3,4-dihydroxyphenylacetic, hydroxycaffeic, hydroxyphenylacetic, *m*-coumaric, and methoxyphenylacetic acids from Sigma Chemical Co. (St. Louis, MO); and oleuropeine from Roth (Karlsruhe, Germany). (3,4-Dihydroxyphenyl)ethanol was prepared from oleuropeine according to the method described by Graciani Constante and Vázquez Roncero (1980). Oleuropeine aglycone was obtained through the enzymatic hydrolysis of oleuropeine as described by Walter, Fleming, and Etchells (1973) and by applying the purification method illustrated by Montedoro, Servili, Balbioli, and Miniati (1992). Pure HPLC solvents were used in all cases.

2.3. Extraction and determination of phenolic compounds

The phenolic compounds of the virgin olive oils were extracted and purified according to the method described by Cortesi, Ponziani, and Fedeli (1981). The total phenols, expressed as gallic acid (mg/kg), were determined colorimetrically at 765 nm using Folin–Ciocalteau reagent as described by Favati, Caporale, and Bertuccioli (1994). Qualitative and quantitative evaluations of phenolic compounds were carried out by analysing the phenolic extracts with HPLC using gallic acid as an internal standard. The HPLC system was composed of a Beckman chromatograph equipped with a 250×4.6 mm C_{18} Ultrasphere-ODS column; the eluates were detected at 278 nm. The mobile phase used was 2% acetic acid in (A) water vs (B) methanol. The elution gradient applied at a flow rate of 1 ml min^{-1} was: 95% A/5% B for 3 min, 80%A/20%B in 15 min and isocratic for 2 min, 60%A/40%B in 10 min, 50%A/50%B in 10 min, 100%B in 10 min until the end of the run. Samples were dissolved in methanol, and 10 μl of this solution was injected into the column. This method has a good repeatability, since several analyses carried out on the same sample produce a repeatability coefficient of per cent variation (CV%) of 7%, which is similar to the values reported by other Authors (Angerosa, D'Alessandro, Konstantinou, & Di Giacinto, 1995).

2.4. Determination of fatty acid composition

The gas chromatographic analyses of fatty acid methyl esters (Official Journal of the European Communities, 1991) was performed with a Fisons HRGC mega 2 series gas chromatograph (Milan, Italy) with a flame-ionisation detector and equipped with a WCOT fused silica capillary column, FFAP-CB coating, film thickness 0.30 μm, 25 m in length×0.32 mm i.d. from Chrompack (Middleburg, The Netherlands). Oven temperature was isothermal at 180°C, while the temperature of the split–splitless injector was 270°C with a splitting ratio of 1:17 and detector temperature 300°C. The carrier gas was hydrogen at a flow rate of 2 ml min^{-1}.

2.5. Autoxidation stability

The resistance to auto-oxidation of the oils was carried out using the Rancimat apparatus (Methrohm Co., Basel, Switzerland) at 120°C with an air flow of 20 l/h. The results are expressed as induction time (h).

2.6. Statistics

One-way analysis of variance (ANOVA) was used to compare the experimental data that were broken down according to the grinding procedure used.

3. Results and discussion

Based on the analytical indices obtained, the oils examined were defined as belonging to the commercial class of *extra virgin* olive oil. The values obtained employing the official analytical methods (Official Journal of the European Communities, 1991) are not reported in the paper because no substantial difference was noticed which was related to the olive paste preparation technique or to the two cultivars considered.

The fatty acid compositions of the two types of oils are reported in Table 1. The saturated/unsaturated fatty acid ratio is greater in the *Ogliarola Salentina* oil while the monounsaturated/polyunsaturated fatty acid ratio is higher in the *Coratina* oil. This means that, although the *Ogliarola Salentina* oil contains more saturated fatty acids, it also contains a greater amount of polyunsaturated fatty acids.

Table 1
Per cent fatty acid composition of the virgin olive oils of (A) *Ogliarola Salentina* cultivar and (B) *Coratina* cultivar

	A	B
$C_{14:0}$	0.01	0.01
$C_{16:0}$	14.63	8.69
$C_{16:1}$	1.88	0.32
$C_{17:0}$	0.10	0.13
$C_{17:1}$	tr[a]	0.03
$C_{18:0}$	1.76	2.04
$C_{18:1}$	71.70	81.79
$C_{18:2}$	8.67	5.66
$C_{18:3}$	0.62	0.62
$C_{20:0}$	0.31	0.36
$C_{20:1}$	0.32	0.35
$C_{22:0}$	tr	tr
$C_{24:0}$	tr	tr
ΣSaturated/ Σunsaturated	0.20	0.13
ΣMonounsaturated/ Σpolyunsaturated	7.95	13.14

The data reported are means of four independent replicate analyses.
[a] tr = traces (not integrated).

Table 2
Standard phenolic compounds: retention time (RT), relative response factors to gallic acid (RRF) and coefficient of per cent variation (CV%)

Phenolic compounds	RT	RRF	
		Mean[a]	CV%
(3,4-Dihydroxyphenyl)ethanol	12.80	1.63	0.55
Protocatechuic acid	13.56	1.29	1.77
3,4-Dihydroxyphenylacetic acid	15.24	2.33	2.89
(*p*-Hydroxyphenyl)ethanol	18.87	2.24	3.88
p-Hydroxybenzoic acid	19.56	0.95	2.36
Hydroxycaffeic acid	21.11	1.85	2.65
p-Hydroxyphenylacetic acid	21.59	4.15	4.01
Chlorogenic acid	23.59	1.32	2.46
Vanillic acid	24.80	0.72	3.05
Caffeic acid	26.33	0.62	2.51
Syringic acid	28.86	0.41	2.67
p-Coumaric acid	33.82	0.38	2.35
Ferulic acid	35.69	0.53	2.35
Sinapic acid	36.22	1.41	0.83
m-Coumaric acid	36.97	0.21	2.64
p-Methoxyphenylacetic acid	37.53	3.06	2.13
o-Coumaric acid	39.89	0.28	2.91
Oleuropeine	42.04	6.08	2.62
Cinnamic acid	46.35	0.20	2.71
Oleuropeine aglycone	47.80	–	–
Apigenin	49.70	1.33	1.93

[a] $n = 5$.

Table 2 contains a list of the phenolic compounds used as standards with their retention times, relative response factors to gallic acid and coefficients of per cent variation obtained from five replicate analyses. Only the retention time is reported for oleuropeine aglycone.

Fig. 1 depicts the HPLC elution profiles of the phenol extracts from (A) *Ogliarola Salentina* and (B) *Coratina*.

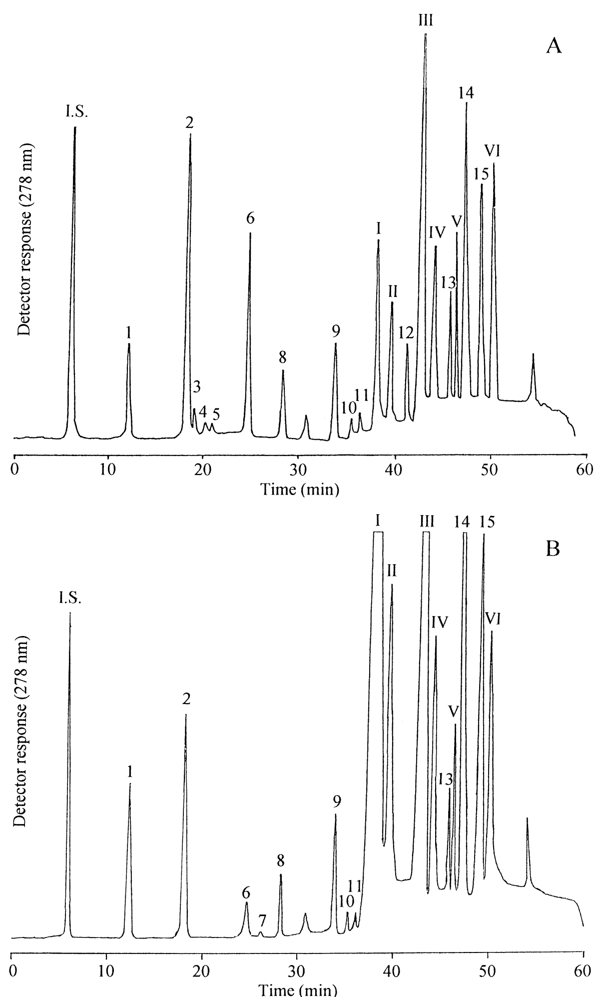

Fig. 1. HPLC elution profiles of the phenol extracts from virgin olive oils: (A) *Ogliarola Salentina,* (B) *Coratina.* I.S., gallic acid, 1. (3,4-dihydroxyphenyl)ethanol; 2. (*p*-hydroxyphenyl)ethanol; 3. *p*-hydroxybenzoic acid; 4. hydroxycaffeic acid; 5. *p*-hydroxyphenylacetic acid; 6. vanillic acid; 7. caffeic acid; 8. syringic acid; 9. *p*-coumaric acid; 10. ferulic acid; 11. *m*-coumaric acid, I. RT = 38.3; II. RT = 39.5; 12. oleuropeine, III. RT = 42.7, IV. RT = 43.6; 13. cinnamic acid, V. RT = 46.5; 14. oleuropeine aglycone; 15. apigenin, VI. RT = 50.7.

All the chromatograms for each cultivar were similar, with differences relating only to the peak areas and mainly ascribable to the different olive paste preparation technique used. The peaks were identified by comparing the retention time of each peak with that of the corresponding standard (Table 2). The peaks that were more difficult to identify were verified by adding the corresponding standards. In all the chromatograms, six unidentified peaks were also present, which are indicated in Roman numerals. According to the literature, they are believed to correspond to at least six phenolic compounds (Solinas, 1987; Montedoro, Servili, Baldioli, Selvaggini, Miniati, & Macchioni, 1993) and, in any case, to simple or hydrolysable phenolic compounds.

Table 3 shows the induction times (h at 120°C), total phenols, and their qualitative and quantitative

Table 3
Analytical composition of the virgin olive oils indicated as mean and SD ($n=10$) of (A) *Ogliarola Salentina* cultivar and (B) *Coratina* cultivar

Determination	Stone mill				Stone mill + kneader				Hammer crusher				Hammer crusher + kneader			
	A		B		A		B		A		B		A		B	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total phenols (mg/kg)	228	28	352	71	175	32	387	50	373	38	490	68	272	44	455	58
Induction time (h at 120°C)	8.7	0.9	14.1	0.7	6.3	1.0	16.1	1.0	11.8	0.7	17.2	0.9	9.1	1.0	16.4	1.1
Phenolic compounds (mg/kg)																
Fraction 1																
(3,4-Dihydroxyphenyl)ethanol	1.17	0.36	1.34	0.20	0.94	0.17	1.72	0.27	1.62	0.26	1.08	0.38	1.48	0.27	1.27	0.35
(*p*-Hydroxyphenyl)ethanol	7.34	1.82	2.95	0.34	9.64	2.57	4.18	0.67	4.68	1.17	1.52	0.39	5.49	1.26	2.37	0.51
p-Hydroxybenzoic acid	0.06	0.04	–	–	0.08	0.04	–	–	0.01	0.01	–	–	0.14	0.08	–	–
Hydroxycaffeic acid	0.06	0.03	–	–	0.11	0.07	–	–	0.04	0.03	–	–	0.23	0.16	–	–
p-Hydroxyphenylacetic acid	0.18	0.11	–	–	0.41	0.19	–	–	0.11	0.09	–	–	0.87	0.27	–	–
Vanillic acid	1.48	0.47	0.32	0.08	1.37	0.42	0.25	0.08	0.76	0.24	0.25	0.05	1.15	0.28	0.23	0.08
Caffeic acid	–	–	0.04	0.01	–	–	0.03	0.02	–	–	0.04	0.01	–	–	0.04	0.02
Syringic acid	0.69	0.27	0.29	0.07	0.62	0.22	0.59	0.22	0.04	0.02	0.09	0.03	0.11	0.08	0.11	0.05
p-Coumaric acid	0.32	0.08	0.36	0.06	0.28	0.08	0.39	0.12	0.14	0.08	0.44	0.10	0.25	0.10	0.38	0.08
Ferulic acid	0.07	0.03	0.42	0.12	0.19	0.11	0.34	0.16	0.01	0.02	0.50	0.14	0.04	0.05	0.34	0.11
m-Coumaric acid	0.05	0.04	0.05	0.01	0.10	0.06	0.07	0.03	0.01	0.01	0.04	0.01	0.01	0.01	0.03	0.02
Cinnamic acid	0.24	0.17	0.16	0.04	0.04	0.04	0.20	0.05	0.51	0.11	0.26	0.05	0.09	0.07	0.29	0.05
Total fraction 1	11.73	2.40	5.91	0.63	13.82	2.89	7.76	0.98	7.93	1.16	4.22	0.65	9.90	1.60	5.04	0.78
Fraction 1/total phenols (%)	5.1	0.8	1.7	0.3	8.1	1.9	2.0	0.2	2.1	0.4	0.9	0.2	3.7	0.9	1.2	0.3
Fraction 2																
Peak I (RT 38.3)	11.53	2.00	67.70	6.51	7.56	1.47	77.77	8.67	17.91	2.32	82.82	6.38	14.88	2.54	76.92	7.35
Peak II (RT 39.5)	4.65	1.70	22.82	7.83	2.65	1.29	12.71	4.35	29.86	11.54	36.68	9.89	20.67	6.17	25.57	9.29
Oleuropeine	4.70	1.03	–	–	3.22	1.09	–	–	2.04	0.94	–	–	1.28	0.65	–	–
Peak III (RT 42.7)	18.28	4.13	73.36	10.56	20.86	4.07	73.78	15.09	21.23	3.14	77.36	7.76	26.11	3.53	76.92	8.63
Peak IV (RT 43.6)	5.33	1.06	19.71	4.52	3.47	0.74	11.05	3.18	13.64	2.96	29.10	9.71	10.31	3.37	20.68	6.48
Peak V (RT 46.5)	5.19	1.43	4.64	1.51	3.99	1.17	5.16	2.11	11.41	3.13	9.14	3.86	10.06	3.49	9.88	5.53
Oleuropeine aglycone	16.17	5.82	32.96	4.87	11.28	3.09	29.78	5.23	27.62	10.45	40.39	3.70	18.64	5.33	36.95	4.90
Apigenin	15.80	4.51	30.87	4.72	11.92	4.89	32.06	4.40	21.03	3.61	28.41	3.05	19.86	3.95	30.37	3.52
Peak VI (RT 50.7)	10.67	2.40	11.86	2.04	11.86	2.30	13.90	3.39	8.63	3.63	11.95	2.15	11.72	4.93	13.20	3.84
Total fraction 2	92.30	10.67	263.81	33.83	76.80	10.20	256.22	34.62	151.32	18.42	315.84	37.45	132.24	16.40	290.56	41.79

composition (mg/kg) for the (A) *Ogliarola Salentina* and (B) *Coratina* oils. All the values obtained in the experiments have been divided according to the paste preparation technique used and are reported synthetically as mean values with their standard deviations.

The oils obtained from the *Coratina* olives contained 60% more total phenols on average (352–490 mg/kg) than the oils obtained from the *Ogliarola* drupes (175–373 mg/kg). In any case, hammer crushers were more apt to extract greater amounts of total phenols than stone mills in both cultivars. Kneading of the olive pastes always produced a reduction in the total phenol content in the *Ogliarola* oils (25% on average); by contrast, the trend of total phenols due to kneading in the *Coratina* oils could not be generalized because increases and decreases were found of the order of less than 10%.

The Rancimat induction times (h at 120°C) were 80% higher on average for the *Coratina* oils (14.1–17.2 h) than for the *Ogliarola* oils (6.3–11.8 h). The values showed the same trends as the total phenols for both grinding techniques and the kneading process.

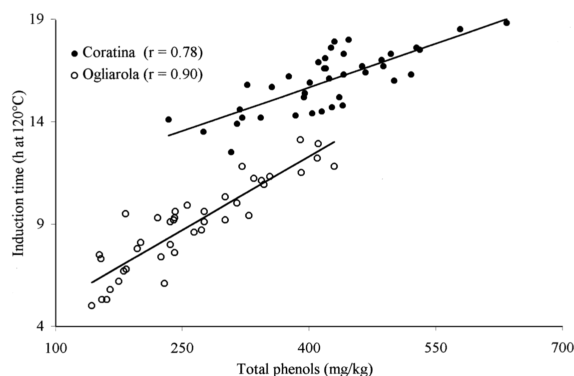

Fig. 2. Correlation between total phenols and induction times.

Fig. 2 relates the total phenols data to the corresponding induction times for the oils of the two cultivars. The overall values show that a direct correlation exists between the two parameters. The regression lines computed for the two types of oils yielded $r=0.78$ ($p<0.001$) for the *Coratina* oils and $r=0.90$ ($p<0.001$) for the *Ogliarola* oils.

The phenol constituents reported in Table 3 were grouped into two fractions. Fraction 1 comprised all the simple compounds and fraction 2 comprised the hydrolysable phenolic compounds and the unidentified peaks. The quantitative analysis was performed by considering the relative response factors to gallic acid for the known substances and a response factor of one (arbitrary) for the oleuropeine aglycone and the unidentified peaks.

On average, fraction 1 never exceeded 8% of the corresponding total phenols. It was present in greater quantities in the oils extracted from the *Ogliarola Salentina* olives (7.93–13.82 mg/kg) than in the oils from the *Coratina* olives (4.22–7.76 mg/kg). On the basis of the induction times we found, most of the simple phenols of fraction 1 may be said to have little or no antioxidant properties, as demostrated by other Authors (Servili & Montedoro, 1989; Papadopoulos & Boskou, 1991). The components more substantially represented in this fraction were (3,4-dihydroxyphenyl)ethanol, (*p*-hydroxyphenyl)ethanol, and vanillic acid—the latter solely in the *Ogliarola* oils. The other simple phenols never exceeded 1 mg/kg in the oils from both cultivars. Some phenolic acids, such as *p*-hydroxybenzoic, hydroxycaffeic and *p*-hydroxyphenylacetic acids, were found only in the *Ogliarola* oils while caffeic acid was detected only in the *Coratina* oils. When milling versus crushing were considered, fraction 1 was present in greater quantities in the oils extracted from the milled pastes than in the oils obtained from the crushed pastes. Kneading of the paste always produced an increase in this fraction. This behaviour may be ascribed to the hydrolytic effects acting on the hydrolysable phenolic constituents because of the longer time it takes to prepare the paste. The single phenolic compounds in fraction 1 do not seem to be affected in the same way by the grinding technique used or by the kneading process.

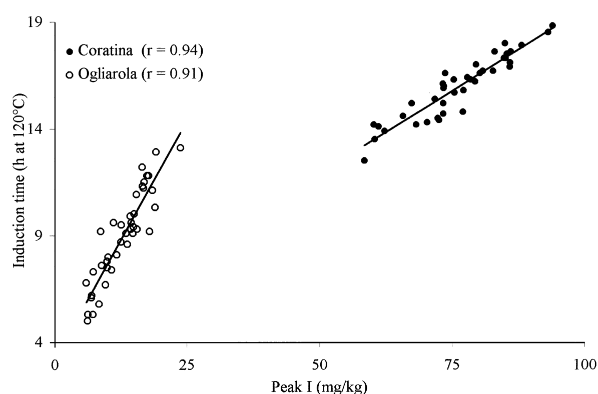

Fig. 3. Correlation between peak 1 (HPLC) and induction times.

Fraction 2 was always present in greater quantities than fraction 1 in both the oils of the two cultivars. In particular, fraction 2 in the *Coratina* oils (256.22–315.84 mg/kg) was practically more than twofold the amounts present in the *Ogliarola Salentina* oils (76.80–151.32 mg/kg). This was true for most of the components of the fraction. The components of fraction 2 may be responsible for the more accentuated ‘bitter-pungent’ taste of the typically ‘bitter’ oils as compared to the typically ‘sweet’ oils. Worthy of note amongst the single, most representative components of this fraction in the oils of both cultivars are: oleuropeine aglycone, and peaks I and III. Twofold greater amounts of oleuropeine aglycone were found in the oils extracted from the *Coratina* olives. Peaks I and III behaved similarly to fraction 2 as greater quantities were present in the *Coratina* oils than in the *Ogliarola* oils. Oleuropeine was always absent in the *Coratina* oils.

Table 4
Variance analysis of some analytical parameters (mean ± SD; $n = 10$) as a function of the different olive grinding techniques used for (A) *Ogliarola Salentina* cultivar and (B) *Coratina* cultivar

Determination	A			B		
	Stone mill + kneader	Hammer crusher + kneader	ANOVA	Stone mill + kneader	Hammer crusher + kneader	ANOVA
Total phenols (mg/kg)	175 ± 32	272 ± 44	$p < 0.001$	387 ± 50	455 ± 58	$p < 0.01$
Induction time (h at 120°C)	6.3 ± 1.0	9.1 ± 1.0	$p < 0.001$	16.1 ± 1.0	16.4 ± 1.1	n.s.
Total fraction 1 (mg/kg)	13.82 ± 2.89	9.90 ± 1.60	$p < 0.01$	7.76 ± 0.98	5.04 ± 0.78	$p < 0.001$
Fraction 2 (mg/kg)						
Peak I	7.56 ± 1.47	14.88 ± 2.54	$p < 0.001$	77.77 ± 8.67	76.92 ± 7.35	n.s.
Peak II	2.65 ± 1.29	20.67 ± 6.17	$p < 0.001$	12.71 ± 4.35	25.57 ± 9.29	$p < 0.001$
Oleuropeine	3.22 ± 1.09	1.28 ± 0.65	$p < 0.001$	–	–	–
Peak III	20.86 ± 4.07	26.11 ± 3.53	$p < 0.01$	73.78 ± 15.09	76.92 ± 8.63	n.s.
Peak IV	3.47 ± 0.74	10.31 ± 3.37	$p < 0.001$	11.05 ± 3.18	20.68 ± 6.48	$p < 0.001$
Peak V	3.99 ± 1.17	10.06 ± 3.49	$p < 0.01$	5.16 ± 2.11	9.88 ± 5.53	$p < 0.05$
Oleuropeine aglycone	11.28 ± 3.09	18.64 ± 5.33	$p < 0.001$	29.78 ± 5.23	36.95 ± 4.90	$p < 0.01$
Apigenin	11.92 ± 4.89	19.86 ± 3.95	$p < 0.001$	32.06 ± 4.40	30.37 ± 3.52	n.s.
Peak VI	11.86 ± 2.30	11.72 ± 4.93	n.s.	13.90 ± 3.39	13.20 ± 3.84	n.s.

When the olive paste preparation techniques were considered, fraction 2 was present in greater quantities in the oils extracted from the crushed pastes than in the oils obtained from the milled pastes. Almost all the components of the fraction behaved in the same way. Kneading of the paste produced a reduction of the amounts of fraction 2 present in the oils. This was also observed for most of the single components of the fraction. For both cultivars, peak I shows the same trend as the total phenols and the induction time.

The behaviour of peak I and the induction times in the oils of both cultivars were further examined by correlating the single values (Fig. 3). The regression lines calculated for the *Coratina* oils and for the *Ogliarola Salentina* oils yielded $r=0.94$ and $r=0.91$, respectively, and $p<0.001$ in both cases. This correlation was greater than the correlation between the induction times and total phenols (Fig. 2), especially in the *Coratina* oils. One would then suppose that the compound of peak I is probably one of the phenolic compounds that make major contributions to preserving the oils.

Table 4 shows the results of the statistical analysis of some of the most representative parameters used in evaluating the variations produced by the different paste preparation techniques. The results are presented separately for the *Ogliarola Salentina* (A) and the *Coratina* (B) oils.

In the *Ogliarola* oils the induction times and the total phenols of the oils obtained from the crushed and kneaded pastes were significantly higher than those of the oils extracted from the milled and kneaded pastes. The whole of fraction 1 was present in scanty quantities and, in any case, it was significantly higher in the milled pastes than in the crushed pastes. Conversely, the components of fraction 2, with the exception of oleuropeine and peak VI, were present in significantly greater amounts in the oils obtained from crushed and kneaded pastes than in the oils extracted from the milled and kneaded pastes.

In the *Coratina* batches, the induction times of the oils obtained from the crushed and kneaded pastes did not differ significantly from those of the oils extracted from the milled and kneaded pastes. When the milling and kneading technique was used, the total phenols, oleuropeine aglycone and peaks II, IV, and V were significantly reduced and this might be responsible for the less 'bitter or pungent' taste of the *Coratina* oils produced in this way.

It appears to be more suitable to use the hammer-crusher plus kneader system to process olives yielding typically 'sweet' oils (e.g. *Ogliarola Salentina*) and to use the stone mill plus kneader system to process olives generally yielding oils with a marked 'bitter to pungent' taste (e.g. *Coratina*). Differentiating the systems would thus be instrumental in achieving the better organoleptic qualities of *extra virgin* olive oils while enhancing their preservation.

4. Conclusions

A significant correlation was found to exist between total phenols and induction times in both the oils, obtained from *Ogliarola* and *Coratina* olives. As for the phenolic compounds analysed by HPLC, they were grouped in two fractions indicated as 1 and 2. Fraction 1 comprised the simple phenols, mainly (3,4-dihydroxyphenyl)ethanol and (*p*-hydroxyphenyl)ethanol and was present in scanty amounts, especially when the paste had been hammer-crushed; it always increased after the paste was kneaded. Fraction 2 consisted of hydrolysable phenols and unidentified peaks and was contained in greater quantities than fraction 1 in both oils. The compound corresponding to peak I showed values which correlated significantly with those of the induction times; it may thus be assumed to be one of the phenolic compounds that make major contributions to preserving the oils. Oleuropeine was found only in the *Ogliarola* oils. The statistical analysis helped determine which technology is more suitable for the characteristics of the olive cultivars, since the aim is that of producing oils of greater quality and value which may also better satisfy consumers.

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