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Secoiridoids, Tocopherols, and Antioxidant Activity of Monovarietal Extra Virgin Olive Oils Extracted from Destoned Fruits

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The effect of olive stone removal before processing on the degradation level, secoiridoid and tocopherol contents, and antioxidant activity of monovarietal extra virgin olive oils (EVOOs) was studied. EVOOs were extracted from olives of the Leccino, Moraiolo, Frantoio, Pendolino, Taggiasca, and Colombaia varieties both in the presence and in the absence of the stones. The degradation level of EVOOs was evaluated by acidity, peroxide number, and spectroscopic indices K_{232} and K_{270}, according to EU regulation. The secoiridoid compounds typical of EVOO, namely, the oleuropein and ligstroside derivatives, hydroxytyrosol, tyrosol, and tocopherols were analyzed by HPLC. The antioxidant activity was evaluated by the xanthine oxidase/xanthine system, generating superoxide radical and hydrogen peroxide, and by the 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl test. Results showed that EVOOs obtained from both stoned and destoned olives had a very low degradation level, which was not affected by destoning. Destoning lowered slightly the α-tocopherol content in EVOOs but increased the total secoiridoid content and the antioxidant activity of EVOOs (up to 3.5-fold). However, these effects were variety-dependent and negligible in some conditions. It was concluded that a better knowledge of the reactions occurring during olive processing, and particularly on the involvement of endogenous pulp and stone enzymes, is essential to predict the effect of destoning on EVOO quality.

KEYWORDS: Extra virgin olive oil; destoning; oleuropein derivatives; ligstroside derivatives; α-tocopherol; 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl; xanthine oxidase

INTRODUCTION

Owing to its unique nutritional and sensory properties, extra virgin olive oil (EVOO) consumption is steadily increasing, both in producing and in nonproducing countries (*1*). The nutritional properties of EVOO have been related to its antioxidant components, such as tocopherols, squalene, and phenolics (*2*, *3*). These latter include secoiridoid aglycons (**Figure 1**) deriving from olive secoiridoid glucosides, which are present only in plants belonging to the Oleaceae family (*4*, *5*).

In all of the Mediterranean countries the more currently used EVOO extraction processes require grinding of the whole olives and malaxation of the paste, followed by separation of the oil from the solids and vegetation water by centrifugation or pressure. The ancient Latin writer Columella (1st century A.D.) described an alternative process for olive oil production, which involves olive destoning before extraction of the oil (*6*). Although destoning lowers olive oil yields, manufacturing plants for stone removal and milling of pulp have been recently set up, showing good oil yield. Interest in this technology is increasing, and some producers believe that oils obtained from destoned olives are of better quality than oils extracted from the whole fruit (*7*). Reports on this issue are contradictory. The first study by Frega et al. (*8*) showed that oils extracted from destoned olives had a greater oxidative stability, as measured by the Rancimat test, and better sensory properties than oils obtained from the traditional milling of entire fruits. Accordingly, Angerosa et al. (*9*) showed that oils obtained from olive pulp had a higher content in volatiles arising from the lipoxygenase pathway than oils obtained by the whole olives. Bentivenga et al. (*10*) identified the presence of α-copaene and α-muurolene in the volatile fraction of an oil extracted from destoned olives, but these compounds were not present in oils obtained from whole olives. Servili and Montedoro (*11*) found that total phenolic concentration can be improved by extracting oil from destoned olives. On the contrary, Patumi et al. (*12*) found no effect of destoning on olive oil quality, as measured by acidity, peroxide number, spectrophotometric indices, phenolics, and volatiles. As documented by these studies, destoning had no influence on the fatty acid and sterol composition of olive oil (*8*, *12*).

It was shown that olive pulp and stone produce two different kinds of oil. With respect to antioxidants, the secoiridoid compounds such as oleuropein and demethyloleuropein are concentrated mainly in the pulp; nuzenide has been detected exclusively in the seed (*13*), and the tocopherol content is higher

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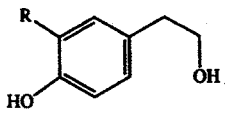
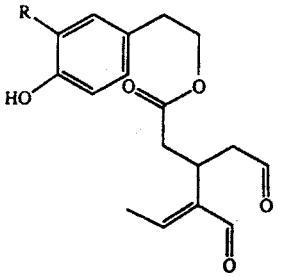
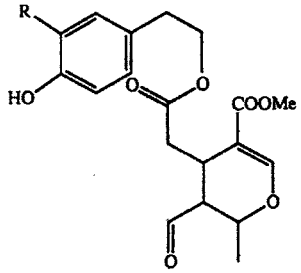


[1] R = OH; CA Index Name: 1,2-Benzenediol, 4-(2-hydroxyethyl)
other name: hydroxytyrosol
[2] R = H; CA Index Name: Benzeneethanol, 4-hydroxy
other name: tyrosol



[3] R = OH; CA Index Name: 4-Hexenoic acid, 4-formyl-
3-(2-oxoethyl)-, 2-(3,4-dihydroxyphenyl)ethyl ester
other name: dialdehydic form of decarboxymethyl oleuropein aglycone

[4] R = H; CA Index Name: 4-Hexenoic acid,
4-formyl-3-(2-oxoethyl)-, 2-(4-hydroxyphenyl)ethyl ester
other name: dialdehydic form of decarboxymethyl ligstroside aglycone



[5] R = OH; CA Index Name: 2H-Pyran-4-acetic acid, 3-formyl-
3,4-dihydro-5-(methoxycarbonyl)-2-methyl-, 2-(3,4-dihydroxyphenyl)ethyl ester
other name: aldehydic form of oleuropein aglycone
[6] R = H; CA Index Name: 2H-Pyran-4-acetic acid, 3-formyl-
3,4-dihydro-5-(methoxycarbonyl)-2-methyl-, 2-(4-hydroxyphenyl)ethyl ester
other name: aldehydic form of ligstroside aglycone

Figure 1. Main phenolic compounds reported in EVOO and discussed in this paper. Possible formation pathways of **3** and **5** from the secoiridoid glucoside oleuropein and of **4** from the secoiridoid glucoside ligstroside have been described elsewhere (*4*, *19*).

in the seed (*14*). However, >97% of olive extractable fatty substance is concentrated in the outer layers of the drupe, whereas the contribution of the seed to the extractable fatty substance is only 3% (*14*). The hypothesis that the effect of the stones on olive oil composition could be associated with its endogenous oxidoreductases (particularly peroxidases), rather than with its extractable fatty substance, has been suggested. These enzymes are believed to catalyze oxidative reactions during malaxation of the olive paste, leading to the degradation of hydrophilic phenols and affecting the oil stability and sensory properties (*8*, *11*). The decrease in concentration of secoiridoid aglycons with increasing time and temperature of malaxation confirms the occurrence of oxidative reactions in this phase (*11*).

The aim of the present work was to evaluate the effect of destoning on some indices of olive oil quality, namely, the degradation level, secoiridoid and tocopherol contents, and antioxidant activity. Antioxidant activity was evaluated by using xanthine oxidase (XOD) as a generator of superoxide anion and hydrogen peroxide, because XOD is one of the main biological catalysts involved in cell damage in vivo (*15*). For comparison, the radical scavenging activity toward the synthetic 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH) was also evaluated. EVOOs were extracted from destoned or stoned fruits of six different olive varieties.

MATERIALS AND METHODS

Oil Samples. Fruits of olive varieties Pendolino and Frantoio were obtained from Il Roccolo, Garda (Verona, Italy); fruits of olive variety Moraiolo were obtained from Premiato Oleificio Vanini Osvaldo, Lenno (Como, Italy); and fruits of olive variety Leccino were obtained from Azienda Agnelli, Bellagio (Como, Italy). Sampling was limited to the period when olives are harvested and processed in the geographic area considered. For each variety a homogeneous group of fruits according to skin color was selected (3 kg), representing the prevalent color of the fruits on the tree. Only healthy fruits, without any kind of infection or physical damage, were selected. Pendolino, Frantoio, and Moraiolo fruits were harvested at the beginning of November and had an epidermis of a black color except for Frantoio fruits, which had an epidermis of a dark green color, because ripening takes place late for this variety. Leccino fruits were harvested at the beginning and at the middle of November (Leccino unripe, U, and Leccino ripe, R, respectively) and had an epidermis of yellowish color with reddish spots and black color. Leccino R olives were separated into two groups, one of which was extracted immediately, and the other was stored for 15 days at 15 °C before extraction to obtain fruits that had been submitted to a postharvest stress (Leccino over-ripe, OR). For each olive sample,

the fruits were separated into two subsamples, one of which was destoned by hand with a knife. The pulp/stone ratios (w/w) for Leccino U, Leccino R, Leccino OR, Frantoio, Pendolino, and Moraiolo were 1.3, 1.3, 1.02, 1.98, 2.2, and 2.75, respectively. Monovarietal EVOOs were produced from stoned and destoned olives in a laboratory-scale oil mill consisting of a hammer crusher, a mixer, and a basket centrifuge. Malaxation was conducted at 30 °C for 40 min. No water was added for oil separation.

Fruits of the Taggiasca and Colombaia varieties were harvested twice at the beginning of November and had an epidermis of a black color. They were processed within the same day at Azienda Agricola Domenico Ruffino, Finale Ligure (Savona, Italy). For each variety and each harvesting day, fruits were separated in two groups, one of which was destoned by a Toscana Enologica Mori (Firenze, Italy) destoner. The pulp/stone ratios (w/w) for Taggiasca and Colombaia were 2.0 and 2.4, respectively. Taggiasca and Colombaia EVOOs were extracted from destoned and stoned olives according to an industrial plan consisting of a metal hammer crusher, a mixer operating at 20−22 °C for 40 min, and a dual-phase decanter (Toscana Enologica Mori, Firenze, Italy). Because analytical data of the two batches of EVOOs extracted on two different days from the same variety and in the same conditions (with or without stones) were not significantly different ($p < 0.05$), only one set has been reported in the tables.

Acidity, peroxide number, and spectroscopic indices K_{232} and K_{270} in the UV region were determined according to the EU official method (*16*).

Phenols. Phenols were determined by HPLC according to the method of Cortesi et al. (*17*). Methanol was used as an extraction solvent. Operating conditions were as follows: 250 × 4.6 mm, 5 μm, RP-18 Spherisorb ODS-2 column (Waters, Vimodrone, Italy) equipped with a Spherisorb ODS-2 precolumn; injection volume, 20 μL; mobile phase, elution with a binary gradient of methanol/acetonitrile (50:50 v/v) (solvent A) and 0.5% H_3PO_4 in water (solvent B), at 1 mL/min. The solvent gradient changed according to the following conditions: from 5% A−95% B to 35% A−70% B in 25 min; to 40% A−60% B in 10 min; to 48% A−52% B in 5 min; to 70% A−30% B in 10 min; to 100% A−0% B in 5 min; to 5% A−95% B in 2 min, followed by 13 min of maintenance. Chromatograms were acquired at 280 nm.

Reference Compounds. Benzeneethanol, 4-hydroxy (**2** in **Figure 1**) was obtained from Merck (Darmstadt, Germany); 1,2-benzenediol, 4-(2-hydroxyethyl (**1** in **Figure 1**) was synthesized according to the method of Montedoro et al. (*4*); 2H-pyran-4-acetic acid, 3-formyl-3,4-dihydro-5-(methoxycarbonyl)-2-methyl-, 2-(3,4-dihydroxyphenyl)ethyl ester (**5** in **Figure 1**) was obtained according to the method of Limirioli et al. (*18*) from oleuropein glycoside (Extrasynthese, Genay, France) by enzymatic reaction using β-glycosidase from almonds (Sigma, St. Louis, MO); 4-hexenoic acid, 4-formyl-3-(2-oxoethyl)-, 2-(3,4-dihydroxyphenyl)ethyl ester (**3** in **Figure 1**) was isolated from olive leaves according to the procedure of Paiva-Martins and Gordon (*19*). 4-Hexenoic acid, 4-formyl-3-(2-oxoethyl)-, 2-(4-hydroxyphenyl)ethyl ester, and 2H-pyran-4-acetic acid, 3-formyl-3,4-dihydro-5-(methoxycarbonyl)-2-methyl-, 2-(4-hydroxyphenyl)ethyl ester (**4** and **6** in **Figure 1**) were identified by a comparison with a reference sample obtained by Prof. G. Fregapane, Departamento de Quimica Analitica y Tecnologia de Alimentos, Facultad de Quimicas, Universitad de Castilla-La Mancha, Ciudad Real, Spain.

Quantification. **1** and **2** contents were calculated by using a standard curve of **2**. **3**−**6** contents were calculated by using a standard curve of oleuropein glycoside.

Tocopherols. Tocopherols were determined by HPLC according to the method of Tonolo and Marzo (*20*). Ethyl acetate was used as an extraction solvent. Operating conditions were as follows: 250 × 4.6 mm, 5 μm, Symmetry C-18 column (Waters, Vimodrone, Italy) equipped with a Symmetry C-18 precolumn; injection volume, 20 μL; mobile phase, isocratic elution with 90:10 methanol/water (94:4 v/v) (solvent A) and ethyl acetate (solvent B) for 20 min, followed by rinsing with 100% B for 7 min, and 90% A−10% B for 13 min, at 1.4 mL/min. Chromatograms were acquired at 294 nm.

Quantification. α-Tocopherol content was calculated by a calibration curve obtained with a commercial standard (Merck).

Antioxidant Activity. The procedure for evaluating the antioxidant activity was reported previously (*21*) and is briefly described as follows.

Extraction of the Polar Fraction. EVOO (2 g) was extracted with methanol (5 mL) or with acetone/water, 90:10 (5 mL). The mixtures were vigorously stirred for 1 h at room temperature and then centrifuged (4500*g* at 15 °C for 10 min) to separate the polar and the lipid fractions. Methanol extracts were used to measure the antioxidant activity by the DPPH test. Acetone/water, 90:10, extracts were used to measure the antioxidant activity when using the XOD/xanthine, as methanol itself inhibits XOD.

XOD/Xanthine System. This system contained 0.1 M phosphate buffer, pH 7.4, 0.5 mM xanthine (in 10 mM NaOH), 0.08 U XOD (from cow's milk, Roche, Monza, Italy), 1.25 mM butanoic acid, 4-(methylthio)-2-oxo, and different dilutions of acetone/water, 90:10, extract of EVOO. The final acetone concentration in the assay mixture was kept constant at 2.5% (v/v). The reaction was carried out at 37 °C for 30 min, followed by gas chromatographic determination of ethene released from butanoic acid, 4-(methylthio)-2-oxo. Control reaction was prepared by adding the extraction solvent (acetone/water, 90:10) in place of the antioxidant extract. The percent inhibition of the control reaction rate was calculated, and a dose−response curve was constructed. The antioxidant activity was expressed as I_{30}, as interpolated by the linear tract of the dose−response curves (10−40% inhibition). The I_{30} is the amount of original oil sample (in milligrams) that caused 30% inhibition of the model reaction under the conditions described above.

DPPH Scavenging Test. Different dilutions of the methanolic extracts of EVOO were added to a 25 mg/L methanolic solution of DPPH (Sigma). The decrease in absorbance was determined at 515 nm after 15 min (when a constant value was reached). The percent decrease of DPPH concentration was calculated, and a dose−response curve was constructed. The DPPH scavenging activity was expressed as I_{50}, as interpolated by the dose−response curve (linear in the range 10−90% inhibition). The I_{50} was defined as the amount of original oil sample (in milligrams) required to lower the initial DPPH concentration by 50%.

HPLC Equipment. The HPLC equipment consisted of an L-7100 Merck Hitachi pump, an L-7400 Merck Hitachi UV−vis detector, and a D-7500 Merck Hitachi integrator.

GC Equipment. The GC equipment consisted of a Varian Aerograph 3300 with a Varian integrator and a 100 cm × 1/8 in. deactivated aluminum oxide column: column temperature, 60 °C; injection temperature, 80 °C; FID temperature, 225 °C.

UV−Vis Spectrophotometer. UV−vis measurements were performed with a Jasco UVDEC-610 spectrophotometer.

Statistical Analysis. Data represent the mean duplicate analysis with analysis of variance conducted with Statgraphics 5.1 (STCC Inc., Rockville, MD); Tukey's honestly significant differenence (HSD) procedure ($p < 0.05$) was used to discriminate among the means.

RESULTS AND DISCUSSION

The degradation levels of EVOOs were evaluated by acidity, peroxide value, and the spectroscopic indices K_{232} and K_{270}, according to EU Regulation 1989/2003 (*22*), and are reported in **Table 1**. It was found that for the EVOOs obtained both in a laboratory-scale oil mill and at a factory, the degradation levels were not significantly affected by the presence or absence of the stones during extraction, but depended on olive quality. For all EVOOs acidity, peroxide value, and K_{232} were markedly below the limits fixed by the EU regulation for olive oil to be labeled as "extra virgin" (*22*). On the contrary, K_{270} was near the legal limit, except for Leccino EVOOs. In agreement with these data it was reported that the K_{270} of some fresh EVOOs approached the threshold established by the EU regulation for the highest quality oil category (*23*).

The antioxidant composition of EVOO is the result of a number of variables acting before oil extraction (such as olive variety, environmental, climatic, soil and cultivation conditions, olive ripeness, olive health) and during EVOO extraction and

Table 1. Acidity, Peroxide Value, Spectroscopic Indices K_{232} and K_{270}, and α-Tocopherol Content of EVOOs[a]

olive	acidity (oleic acid %)	peroxide value (mequiv of O_2/kg)	K_{232}	K_{270}	α-tocopherol (mg/kg) stoned	α-tocopherol (mg/kg) destoned
Leccino U	0.14ab	3.0b	1.42a	0.12ab	474y,f	419z,e
Leccino R	0.14ab	2.9b	1.43a	0.12ab	355y,e	334z,d
Leccino OR	0.12a	2.1a	1.39a	0.10a	348y,e	326z,d
Frantoio	0.16bc	3.4bc	1.74c	0.18ab	174y,b	151z,b
Pendolino	0.19cd	3.7c	1.56b	0.17ab	215z,c	212z,c
Moraiolo	0.16bc	2.2a	1.66bc	0.18ab	243z,d	231z,c
Taggiasca	0.16bc	4.5d	1.68c	0.18ab	188y,b	165z,b
Colombaia	0.22d	4.7d	1.76c	0.19b	81z,a	74z,a
legal limit (EU Regulation 1989/2003)	≤0.8	≤20	≤2.5	≤0.22		

[a] Different letters within a column (a–f) indicate significant differences ($p < 0.05$) with respect to olive composition. Different letters within a row (z, y) indicate significant differences ($p < 0.05$) in α-tocopherol content with respect to destoning. Destoning did not significantly affect EVOO degradation level ($p < 0.05$). $n = 2$.

Table 2. Phenolic Content of EVOOs[a]

	total (mg/kg)		**3** (mg/kg)		**4** (mg/kg)		**5** (mg/kg)		**6** (mg/kg)		**1** (mg/kg)		**2** (mg/kg)	
olive	stoned	destoned	stoned	destoned	stoned	destoned	stoned	destoned	stoned	destoned	stoned	destoned	stoned	destoned
Leccino U	325z,bc	886y,c	159z,c	659y,e	116z,c	162y,c	24z,a	35y,a	24z,abc	28z,ab	0.35z,a	0.46z,a	1.9z,ab	1.3z,a
Leccino R	429z,d	1241y,d	227z,d	942y,f	150z,d	226y,d	28z,a	41y,ab	21z,ab	28y,ab	0.46z,a	0.57z,a	2.7z,b	3.0z,b
Leccino OR	106z,a	373y,a	14z,a	185y,ab	45z,a	100y,a	23z,a	46y,b	22z,ab	40y,bc	0.55z,a	0.62z,a	1.2z,a	1.7z,a
Frantoio	311z,b	529y,b	155z,c	337y,c	113z,c	133y,b	26z,a	39y,ab	15z,a	19z,a	0.53z,a	0.46z,a	1.5z,a	1.0z,a
Pendolino	502z,d	637y,b	329z,e	448y,d	87z,b	94z,a	54z,b	58z,c	28z,bc	32z,bc	1.3z,a	1.6z,b	2.9z,b	3.4z,b
Moraiolo	1072z,e	1115z,d	823z,f	866z,f	123y,c	100z,a	89z,c	101y,e	34z,c	42z,c	1.0z,a	1.3z,ab	1.8z,ab	1.2z,a
Taggiasca	423z,cd	522y,b	169z,cd	214y,b	199z,e	230y,d	nd	nd	25z,abc	68y,d	10.7y,c	4.6z,c	19.7y,c	5.5z,c
Colombaia	437y,d	335z,a	88z,b	85z,a	221y,f	134z,b	47z,b	68y,d	52y,d	44z,c	9.3y,b	0.9z,ab	20.0y,c	2.9z,b

[a] Different letters within a column (a–f) indicate significant differences ($p < 0.05$) with respect to olive composition. Different letters within a row (z, y) indicate significant differences ($p < 0.05$) with respect to destoning. $n = 2$.

storage (*24*, *25*). The main tocopherol compound in EVOOs is α-tocopherol. As shown in **Table 1**, the α-tocopherol content of EVOOs was affected both on olive composition and on destoning. With respect to olive composition, EVOOs extracted form unripe olives of Leccino variety had the highest α-tocopherol content (474 mg/kg in the presence of stone and 419 mg/kg in the absence of stone). The α-tocopherol content decreased in EVOOs extracted from ripe Leccino olives (355 mg/kg in the presence of stone and 334 mg/kg in the absence of stone), but it was not affected by over-ripening. Colombaia EVOOs had ∼6-fold lower α-tocopherol content with respect to Leccino EVOOs (81 mg/kg in the presence of stone and 74 mg/kg in the absence of stone), and the other EVOOs had intermediate contents. In general, destoning lowered the α-tocopherol content in all EVOOs, in agreement with results reported by Frega et al. (*8*). However, the effect of this technology was negligible if compared with genetic factors and with the effect of ripening.

The main olive secoiridoid glucosides are not present in EVOO because of their high water solubility. However, during the crushing of olives oil-phase-soluble derivatives arise from the chemical and enzymatic hydrolysis of secoiridoid glucosides (*26*). **3**−**5** have been identified in EVOOs (*4*). By analogy with **5**, a structure has been proposed for **6** (*5*). Phenyl acids and phenyl alcohols including **1** and **2** are present in low amounts in fresh EVOOs, and their content increases during storage of EVOOs, probably due to the hydrolysis of **3**−**6** (*24*).

As shown in **Table 2**, the phenolic content of EVOOs was affected both by olive composition and on destoning. Among EVOOs extracted from stoned olives the highest phenolic content was found in Moraiolo EVOO (1072 mg/kg); Pendolino, Colombaia, Leccino R, and Taggiasca EVOOs had about half of this value. Lower contents were observed in Frantoio EVOO (311 mg/kg) and in Leccino EVOOs extracted from early-harvested stoned olives (325 mg/kg) and over-ripe stoned olives (106 mg/kg). Destoning caused an increase in the phenolic contents of all EVOOs, except for EVOOs extracted from Colombaia and Moraiolo varieties. As a result, among EVOOs extracted from destoned olives, Moraiolo and Leccino R EVOOs had the highest phenolic contents (∼1200 mg/kg), followed by Leccino U EVOO (886 mg/kg), Pendolino, Frantoio, and Taggiasca EVOOs (522−637 mg/kg), and Leccino OR and Colombaia EVOOs (∼350 mg/kg). With respect to individual components, destoning increased the content of **3** in all EVOOs, except for those extracted from Moraiolo and Colombaia olives, which had similar contents regardless of the presence of the stone. The observed increase was dependent on olive variety and ripening stage: Leccino EVOO extracted from over-ripe olives showed the highest increase (13-fold), followed by Leccino EVOOs extracted from unripe and ripe olives of Leccino variety (4-fold), Frantoio EVOO (2-fold), and Pendolino and Taggiasca EVOOs (1.3-fold). The contents of the other phenolic compounds showed lower variations due to destoning. The contents of **5** increased only slightly in all EVOOs obtained from destoned olives, except for that extracted from Pendolino. The contents of **4** increased only slightly in all EVOOs obtained from destoned olives, except for those extracted from Pendolino, Moraiolo, and Colombaia. The content of **6** did not show a clear trend of variation due to destoning. The content of **1** and **2** decreased in Taggiasca and Colombaia EVOOs extracted from destoned olives, but in the other EVOOs they were not affected by destoning.

A previous work has shown that the oxidative stability of EVOOs as measured by Rancimat test increased by 10-fold in EVOOs extracted from destoned olives with respect to EVOOs extracted from the whole fruit. This effect was attributed to a

Table 3. Antioxidant Activity of EVOOs[a]

	DPPH (I_{50}, mg of oil)		XOD/xanthine (I_{30}, mg of oil)	
olive	stoned	destoned	stoned	destoned
Leccino U	53y,d	23z,ab	20.0y,b	6.2z,a
Leccino R	54y,d	20z,a	24.0y,c	6.9z,ab
Leccino OR	133y,e	61z,d	61.0y,d	29z,f
Frantoio	41y,c	20z,a	18.0y,b	10.4z,bc
Pendolino	36y,c	30z,c	17.1y,b	14.9z,de
Moraiolo	22z,a	22z,ab	11.3z,a	12.6z,cd
Taggiasca	30y,b	26z,bc	16.5y,b	14.1z,d
Colombaia	30z,b	31z,c	17.0z,b	18.0z,e

[a] Different letters within a column (a–f) indicate significant differences ($p < 0.05$) with respect to olive composition. Different letters within a row (z, y) indicate significant differences ($p < 0.05$) with respect to destoning.

different content of phenolic compounds. However, no quantitative data on these compounds were provided (*8*). Other studies reported that the phenolic content increased only slightly in EVOOs extracted from destoned olives with respect to EVOOs extracted from the whole fruit or were not affected (*11*, *12*). In general, data reported in **Table 2** show that the effect of destoning on phenolic concentration was different according to the olive variety and ripening stage, and this result may explain the contradictory data reported in the literature (*8*, *11*, *12*).

Among EVOO phenolics, oleuropein derivatives, which share an *o*-diphenolic structure, are mainly responsible for the effects of EVOO on cells, which are potentially indicative of possible health effects, rather than ligstroside derivatives, as studied in vitro by different cell or cell-free systems (*2*, *3*, *21*, *27*, *28*). Due to XOD involvement in the oxidative stress occurring in vivo, the XOD-catalyzed reaction has been used to study the properties of different phytochemicals and food extracts (*15*, *29*, *30*). It has been found that cell damage due to the XOD-catalyzed reaction can be prevented by both **1** and oleuropein (*28*, *31*). We reported previously that also EVOO extracts inhibited the XOD-catalyzed reaction and had scavenging activity toward the DPPH radical. The efficacy of XOD inhibition and DPPH scavenging of EVOO extracts showed a linear correlation with the content of *o*-diphenolic compounds (*21*).

On the basis of these studies we investigated the effect of destoning on EVOO antioxidant activity. As shown in **Table 3**, the antioxidant activity of EVOOs, measured as the ability to inhibit XOD, depended both on genetic factors and on the ripening stage, as well as on the presence or absence of the stone during oil extraction. The variation of the DPPH radical scavenging ability of EVOOs showed similar behavior. As observed for the changes in the contents of **3**, the changes in the antioxidant activity due to destoning were variety-dependent. In particular, the antioxidant activity of EVOOs extracted from Leccino and Frantoio varieties showed the highest increase in antioxidant activity after destoning (up to 3.5-fold). EVOOs extracted from Pendolino and Taggiasca olives showed a moderate increase in antioxidant activity after destoning (1.2-fold), whereas the antioxidant activities of EVOOs extracted from Moraiolo and Colombaia olives were not affected by destoning.

The present study has shown that olive destoning before processing can enhance the phenolic content and antioxidant activity of EVOO. The content of **3**, which is the main oleuropein derivative in EVOO and a powerful antioxidant and could account for the observed healthful effects of EVOO consumption (*2*, *3*), was particularly increased by destoning. These results indicate that destoning may improve the nutritional properties of EVOO. However, the present study also showed that the effects of destoning were variety-dependent and negligible in some conditions. All of the olives used for the present research came from northern Italy. To achieve significant information on the behavior of olive varieties, geographical, geological, and climatic factors should be taken into consideration together with harvesting time. It is also necessary to point out that in the present research the performance of one industrial oil mill operating olive destoning was studied. To ascertain whether the destoning technology, as suggested by a number of manufacturers, could lead to a significant enhancement of oil quality, other destoning equipment design should be investigated. The overall results are consistent with the hypothesis that stone-related changes could be associated with its endogenous enzymes, as previously proposed (*11*). It may be concluded that a better knowledge of the endogenous enzymes in the olive and of their role during processing is essential to predict the effect of destoning on EVOO quality.

ABBREVIATIONS USED

DPPH, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl; EVOO, extra virgin olive oil; OR, over-ripe; R, ripe; U, unripe; XOD, xanthine oxidase.

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