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ORIGINAL PAPER

Evaluation of Functional Phytochemicals in Destoned Virgin Olive Oil

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Abstract Processing destoned olives by means of extracting adjuvants micronised food talc and depolymerising *Cytolase 0* enzyme complex have been studied in the present work. This innovative processing technology increased the plant efficiency (amounts of olives processed per hour) by 20%, as well as the nutritional quality of the end product with respect to functional compounds. The oils showed higher contents of biophenols, aromas, and tocopherols. An intense and balanced flavor and a potentially higher stability and endurance to oxidation (shelf-life) was found. Contents of chloroplast pigments (chlorophylls, pheophytins, carotenes, and xanthophylls) appeared to be lower in comparison to conventional processing. The processing aids allowed to increase significantly the oil yields and to reduce the oil percentage in the byproducts. Traceability of the new products was still possible applying chemometric data analysis for discriminating between cultivars.

Keywords Destoned oils · Bioactive components · Flavor and aroma compounds · Chemometrics · Traceability

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Introduction

In the last few years, much attention has been paid to the destoned olive processing technique because of improved qualitative features of the resulting products (Servili et al. 2007). Columella and other ancient writers have already supported this theory (Amirante et al. 2006). Consistently, the Italian Ministry for Agricultural Food and Forestry Policies has recently approved some special research projects concerning this current topic. In fact, the new extracting technology could actually contribute to enhance the oil sector competitiveness (Del Caro et al. 2006; Muzzalupo et al. 2007).

Olive seed oil is characterized by an analytical composition similar to that of other seed oils, and it is of inferior quality with respect to olive pulp oil. It is richer in poly unsaturated fatty acids (PUFA) (17.0% vs 8.6%), because of higher percentages of linoleic acid ($C_{18:2}\omega_6$), and decreased percentage of in mono unsaturated fatty acids (69.1% vs 74.7%) and in stearic fatty acids (13.9% vs 16.7%), owing to lower contents of both oleic acid ($C_{18:1}\omega_9$) and palmitic acid. Consequently, its triacylglycerol composition is mainly characterized by lower percentages of both triolein (34.8% vs 40.5%) and 1,2-dioleoyl-3-palmitoyl-glycerol (16.9% vs 26.0%). However, olive seed (kernel) is a minimal proportion (~2%) of the whole fruit (whereas the proportion of pulp is 85%), so that it does not significantly influence the composition of the total fruit oil (Ranalli et al. 2002a, b).

General aspects of the innovative destoning-based olive processing system can be summarized as follows: (1) destoned oils are richer in functional compounds; (2) olive seed tissues are characterized by high concentrations of endogenous oxidoreductase enzymes (especially peroxidases), which fix the oxygen to both fatty acids and

glycerides, thus degrading them; (3) olive endocarp has a high hardness and olive crushing results in a mechanical and resulting termal stress of olive paste, which reaches temperatures as high as 80–90°C in the crushing room. Such conditions strongly promote thermal oil oxidation processes and result in strong emulsification in the oil phase; (4) byproducts are better industrially exploitable since they are already separated in the oil mill. Furthermore, vegetation water is richer in biophenols, compounds recyclable in chemical, cosmetic, nutraceutical, and agro-food industries. Moreover, the woody part of the olive stone, due to the contained oil, can be employed to obtain furan products or as a fuel (calorific power=4,000 kca/kg).

Kernels are exploitable in pharmaceutical and other industries due to the contents of fat, oleuropein, nüzenide, and squalene. The husk can be used as an ingredient of complex feeds (or foods) or to further recover the contained oil (up to 40%, dry matter basis), although its moisture percentage (>60%) is quite high (Lavelli and Bondesan 2005). The drawback is the reduced draining of the liquid paste phases, owing to the removal of the stone fragments, which are a very efficient draining material. The absence of the angular and sharp stone particles contributing to breaking the uncrushed oleiferous cells during paste malaxation results in decrease of the oil output of about 3–4%. In the process with destoned fruits, the oil contained in these cells cannot be mechanically extracted, thus coming out in the byproducts (Patumi et al. 2003). Therefore, a new method was evaluated in the present study, incorporating exogenous depolymerising enzymes and draining micronized food talc into destoned oily pastes. The above mentioned lower oil outputs are attributable to the use of a conventional destoner machine (in the place of the mechanical crusher) that executes a soft olive grinding but does not sufficiently break the parenchymatic cells. Some authors suggested to carry out a soft olive paste crushing after destoning, by a suitable hammer or disk crusher (finisher) (Del Caro et al. 2006).

Although lower oil yields are obtained when subjecting destoned fruits to the processing, the innovative technology including additives has been introduced in some modern oil mills. The higher processing efficiency (+20%), such being the average stone proportion in the olive fruit, as well as the higher added value and price (up to € 80/kg) and more marked healthy and nutritional properties of the new product are seen as economically advantageous. The product is marketed in little (100–500 ml) beautiful bottles. To enhance further its quality, olives to be destoned are sometimes meticulously harvested at the right maturation degree by means of selective harvesting since fruits do not ripen simultaneously on the tree. Also, such olives are often produced according to organic agriculture methods. However, the contradictory data concerning destoned oils cause some uncertainties (Patumi et al. 2003). The aim of this work was to evaluate the processing method and to characterize the new products, focusing on those components more related with their typicality and bioactivity.

Materials and Methods

Six Italian olive varieties (Caroleo, Coratina, Gentile di Chieti, Dritta, Leccino, and Cipressino) and mixtures of them were processed. The analytical data of oils from two homogenous olive mixtures are used. Their relative varietal composition was as follows: mixture 1: Gentile di Chieti 30%, Caroleo 32%, and Coratina 36%; mixture 2: Dritta 25%, Leccino 33%, and Cipressino 42%. Their relative chemical composition was as follows: mixture 1: moisture 46.1%, oil 22.0%, and solids 31.9%; mixture 2: moisture 49.2%, oil 20.8%, and solids 30.0%. All olive varieties were picked by hand at the industrial maturation degree and were processed by a two-phase horizontal centrifugal decanter operated at 4,000 rpm. Their removal strength from the trees ranged from 460 to 470 g. The destoner used was not coupled to a metal crusher. All machines making up the continuous extraction line were supplied by the Rapanelli firm (Foligno, Perugia, Italy). When processing mixtures were made up of whole olive varieties (controls), a mobile hammer crusher with a sieve size of 6 mm was used in the place of a destoner. Fresh, sound, and healthy drupes from trees grown in Middle Italy and produced according to organic agriculture rules were employed.

The exogenous depolymerising enzyme complex applied (Cytolase 0), (Gist-Brocades, Seclin City, France) is made up of pectolytic, cellulolytic, and hemicellulolytic enzyme species. It has a different relative enzyme composition with respect to similar ones, but its activity is likewise not less than 2,000 units/ml. One unit of activity is defined as the amount of enzyme complex liberating 1 μmol of reducing sugars per minute from pectins. It degrades the uncrushed oleiferous cells and the vegetable colloids (pectins, hemicelluloses, proteins, etc.), emulsifying the minute oil droplets. The oil–water emulsions are removed as well since they also contain an *endopolygalacturonase* enzyme species. In addition, the rheological features of olive paste are improved. Such an enzyme preparation is water-soluble and it thoroughly comes out in the liquid effluent (w/w) during the final step of oily must centrifugation of the extraction cycle (Ranalli et al. 2003, 2004). HTP4 micronized food talc (Montalesio, Sondrio, Italy), a draining processing helper, was simultaneously used.

The oil samples were prepared from whole olives, destoned olives (DO), destoned olives with 2% talc added (DO-T), destoned olives with *Cytolase 0* enzyme (600 units kg^{-1}) added (DO-E), and destoned olives with both talc and

Cytolase 0 added in the same mentioned proportions (DO-E+T). For each processing test, a 300-kg olive sample was employed. The raw material (olives) was first washed and defoliated, then destoned/crushed and finally malaxed for 60 min at 30°C. Olive paste was centrifuged using minimal volumes of lukewarm (30°C) diluting tap water. The obtained oily must was separated into oil and water by means of a vertical centrifuge (operated at 9,000 rpm) discharging automatically the residual solids. Both talc and enzyme complex were added to the oily paste at the start of the beating step. For each experiment, samples of olives, oils, vegetable waters, and husk were drawn for analyses.

Raw material and byproducts were analyzed for both moisture (at 105°C) and oil, using for the latter analysis a Soxhlet apparatus and petroleum ether (40–70°C) as the solvent (Ranalli et al. 2005). Analytical determinations of the oil samples (stored frozen at −20°C until analyzed) were mostly run using the reliable methodologies employed in previous works (Ranalli et al. 2004, 2005). They were fully characterized, evaluating primarily the major functional components and the analytical parameters related to aroma, flavor, stability, and shelf-life.

In particular, volatiles were quantified by a dynamic head-space-high-resolution gas chromatography DHS-HRGC method, using a 25-m-long capillary 20-M carbowax column with a 0.32-mm internal diameter and a 0.20-μm ethylene glycol wall (Nordion, Helsinki, Finland) (Ranalli et al. 2004). Some secoiridoid derivatives, namely, tyrosol and hydroxytyrosol and major ligstroside and oleuropein aglycons (dialdehydic forms of elenolic acid containing either hydroxytyrosol or tyrosol), after extraction with methanol, were analyzed by HRGC. A 25-m-long column with a 0.32-mm internal diameter and a 0.20-μm-thick dimethylpolysiloxane wall (Lab. Service Analitica, Anzola Emilia, Bologna, Italy) was used. Resorcinol (>99% pure) was the internal standard used. Tocopherols were analyzed by high-performance liquid chromatography (HPLC), using a 300-mm direct-phase column with a 3.9-mm internal diameter and a 10-μm-thick M-porasil wall (Waters, Milford, MA, USA). A hexane/propan-2-ol (98.5:1.5, v/v) eluent and a ultraviolet (UV) detector at 292 nm were used (Ranalli et al. 2003).

β-carotene and major xanthophylls were determined spectrophotometrically after separation by thin-layer chromatography, using *N*,*N*-dimethylformamide for the extraction and a mixture of petroleum ether 65–95°C–acetone–diethylamine (10:4:1, v/v/v) as developer (Ranalli et al. 2005). Chlorophylls and pheophytins *a* and *b* (Mg-free chlorophyll derivatives) were determined jointly by a colorimetric method (Ranalli et al. 2002b). Chroma (σ%), brightness (h%), and hue (λd) CIE parameters, from which Naudet's integral color index was assessed, were determined by transmittance measurements (Ranalli et al. 2004).

The composition of triglycerides was determined by HPLC using an LDC_4 100 Ms system equipped with a Shodex RF Se-61 differential refractometer and a chromoject integrator (Thermo Separation Products, Schaumburg, IL, USA). Separation was done as described in Ranalli et al. (2003). Fatty acids, phytosterols, triterpene dialcohols, superior triterpene alcohols, aliphatic long-chain alcohols, diterpene alcohols, and waxes were determined by HRGC methods (Ranalli et al. 2005). Oxidized triglycerides, triglyceride oligopolymers, diglycerides, and total polar compounds were determined by high-performance size exclusion chromatography (Gomes and Caponio 1999). Oxidative stability was evaluated by the established Swift's accelerated test assuming as an oxidation mark the induction time of the peroxidising reactions (Ranalli et al. 2003). Oxidative susceptibility was evaluated according to Cert et al. (1996). UV indices (specific extinctions), k_{232}, k_{270}, and Δk, were determined on an oil sample dissolved in isooctane (Ranalli et al. 2005). Carbonyl index was determined colorimetrically using phloroglucin as a reagent and taking the absorbance values at 540 nm. Free acid content and peroxide value were assessed by titrimetric methods (Ranalli et al. 2002a). Finally, the quantitative descriptive sensory profiling was performed according to the procedure described in Annex XII of EEC Reg. n. 2568/91 and the subsequent modifications (EC Reg. n. 796/02).

The experimental methodology followed was based on a 2×5 factorial design (two olive varieties × five technological treatments). All experiments were run in triplicate. Univariate treatments of the data were performed by two-sided variance analysis. When a significant F value was found, means were separated using Tukey's-Kramer honestly significant difference. Either parametric methods, such as principal component analysis (PCA), hierarchical cluster analysis, and canonical discriminant analysis, or nonparametric methods, such as K nearest neighbor classification and classification and regression tree, were applied for multivariate treatments of the data. The statistical software packages Statistica® (Release 6.0, 1999) (Statsoft, Tulsa, OK, USA), Minitab® (release 15.0, 2006) (Minitab, State College, PA, USA), SPSS® (release 12.0, 2002) (SPSS, Chicago, IL, USA), and Stata® (release 9.0, 2005) (Stata, College Station, TX, USA) were used. Some aspects of the followed experimental methodologies have also been considered by other authors in recent works (Martins et al. 2008; Opara et al. 2008).

Results and Discussion

The analytical data, obtained by assessing more than a hundred independent variables, is in part given in Tables 1 and 2 along with the statistical significances. These data

Table 1 Values of analytical variables in two extravirgin olive oil variety mixtures (EOOVM1 = *Gentile di Chieti* 30%, *Caroleo* 32%, *Coratina* 38%; EOOVM2 = *Dritta* 25%, *Leccino* 33%, *Cipressino* 42%)

Analytical variables	EOOVM1					EOOVM2				
	WO	DO	DO-E	DO-T	DO-E+T	WO	DO	DO-E	DO-T	DO-E+T
Squalene (%)	0.6^a	0.7^a	0.7^a	0.8^a	0.6^a	0.7^a	0.7^a	0.8^a	0.6^a	0.6^a
Secoiridoid aglycons (mg kg^{-1})	43^a	51^b	62^c	48^b	64^c	40^a	47^b	55^c	45^b	56^c
o- diphenolics (mg kg^{-1})	97^a	118^b	135^c	114^b	139^c	87^a	91^b	120^c	96^b	128^c
Tocopherols (α+g) (mg kg^{-1})	121^a	133^b	164^c	136^b	169^c	111^a	115^a	132^b	115^a	128^b
Swift's test (h)	14.8^a	15.5^b	16.7^c	15.2^b	16.3^c	13.0^a	14.2^b	15.7^c	14.2^b	15.9^c
Green aromas (C_6LA+C_6LnA) (mg kg^{-1})	348^a	402^b	423^c	411^b	433^c	323^a	358^b	392^c	364^b	400^c
Oxidative susceptibility ($\times 10^{-2}$)	4.7^a	4.2^a	4.5^a	4.8^a	4.7^a	5.8^a	5.5^a	5.7^a	5.9^a	5.9^a
Chloroplast pigments (mg kg^{-1})	20.3^a	17.5^b	18.8^c	17.0^b	19.0^c	19.0^a	16.4^b	17.5^c	16.0^b	17.2^c
Naudet's color index	17.0^a	15.5^b	16.3^c	15.1^b	16.0^c	16.7^a	15.5^b	15.8^b	15.3^b	15.5^b
OOO+PoPP (%)	44.5^a	44.9^a	44.0^a	45.0^a	49.9^a	36.6^a	35.9^a	36.1^a	35.8^a	36.0^a
SOL+POO (%)	22.4^a	22.0^a	22.0^a	21.9^a	21.9^a	23.8^a	24.1^a	24.6^a	24.0^a	23.7^a
Polyphenols to PUFA ratio	36.8^a	39.5^b	42.2^c	38.9^b	41.8^c	25.7^a	27.3^b	29.1^c	27.0^b	28.4^c
$K_{232}\left(E^{1\%}_{1\,cm}\right)$	1.76^a	1.70^a	1.68^a	1.68^a	1.73^a	1.81^a	1.74^a	1.77^a	1.80^a	1.75^a
$K_{270}\left(E^{1\%}_{1\,cm}\right)$	0.18^a	0.19^a	0.17^a	0.18^a	0.16^a	0.13^a	0.12^a	0.11^a	0.10^a	0.10^a
$\Delta K \times 10^3$ (meq 0_2 kg^{-1})	0	0	−1	−1	0	−1	0	0	−1	−1
Carbonyl index — IWM (E/Y)	3.0^a	2.8^a	2.7^a	2.9^a	3.1^a	2.7^a	2.9^a	2.6^a	3.0^a	3.0^a
Free acidity (% oleic acid)	0.5^a	0.4^a	0.4^a	0.4^a	0.5^a	0.4^a	0.4^a	0.3^a	0.3^a	0.4^a
Sensory scoring	7.7^a	8.3^b	8.8^c	8.2^b	8.7^c	7.3^a	7.7^b	8.0^c	7.7^b	8.1^c

E = enzyme *Cytolase 0*; T = micronized food talc, whole olives (WO. Data are means of three replicates (CVs≤7,5%). Within a row and each variety, values with different superscript letters are statistically different (*Tukey's* honestly significant difference range test, $p \leq 0.05$)

show how destoning enhances the quality level of products. The obtained destoned oils, in fact, were richer in green aromas (from both linoleic and α-linolenic acids, having a *cis-cis*-1,4 pentadiene system) (Table 1). This phenomenon is likely due to a lower inactivation level of the hydroperoxidelyase, an enzyme included among those of the lypoxygenase pathway, which is in part inhibited at a temperature as low as 15°C (Salas and Sánchez 1999).

Table 2 Values of other analytical variables in two extravirgin olive oil variety mixtures (EOOVM1 = *Gentile di Chieti* 30%, *Caroleo* 32%, *Coratina* 38%; EOOVM2 = *Dritta* 25%, *Leccino* 33%, *Cipressino* 42%)

Analytical variables	EOOVM1					EOOVM2				
	WO	DO	DO-E	DO-T	DO-E+T	WO	DO	DO-E	DO-T	DO-E+T
Waxes (C_{40}–C_{46}) (mg kg^{-1})	123^a	118^a	127^a	115^a	130^a	101^a	110^{ab}	115^b	107^{ab}	100^a
Diterpene alcohols (mg kg^{-1})	128^a	133^a	124^a	120^a	121^a	149^a	155^a	143^a	151^a	147^a
Triterpene alcohols (mg kg^{-1})	846^a	863^a	811^a	870^a	857^a	531^a	510^a	521^a	555^a	548^a
Aliphatic alcohols (mg kg^{-1})	146^a	128^a	135^a	137^a	144^a	122^a	133^a	119^a	126^a	129^a
Triterpene dialcohols (mg kg^{-1})	22^a	29^a	20^a	24^a	27^a	31^a	26^a	24^a	25^a	25^a
Phytosterols (mg kg^{-1})	1240^a	1291^b	1298^b	1287^b	1301^b	1181^a	1215^b	1221^b	1218^b	1231^b
Alcoholic index	0.28^a	0.25^a	0.28^a	0.30^a	0.31^a	0.08^a	0.09^a	0.07^a	0.08^a	0.06^a
MUFA (%)	71.8^a	70.5^a	71.1^a	71.2^a	71.4^a	78.0^a	78.4^a	78.9^a	78.7^a	78.6^a
PUFA (%)	11.9^a	12.3^a	12.0^a	11.8^a	12.5^a	6.9^a	6.3^a	6.2^a	6.4^a	6.2^a
SFA (%)	16.3^a	17.2^a	16.9^a	17.0^a	16.1^a	15.1^a	15.3^a	14.9^a	14.9^a	15.2^a
ω_6 to ω_3 ratio	10.3^a	10.1^a	10.4^a	10.3^a	10.2^a	13.2^a	12.9^a	13.0^a	12.8^a	12.8^a
Bitter scoring (median)	5.9^a	3.9^b	4.6^c	3.8^b	4.7^c	4.0^a	2.8^b	3.3^c	2.7^b	3.2^c
Sharp scoring (median)	5.1^a	3.2^b	4.2^c	3.1^b	4.0^c	4.7^a	3.0^b	3.9^c	2.8^b	3.9^c
Astringent scoring (median)	4.5^a	3.0^b	3.8^c	2.9^b	4.0^c	3.7^a	2.5^b	3.0^c	2.6^b	3.1^c
Fruitness scoring (median)	6.5^a	7.2^b	8.0^c	7.3^b	7.9^c	6.1^a	6.8^b	7.5^c	6.9^b	7.3^c
Turbidity (NTU)	31^a	17^b	15^b	15^b	17^b	29^a	14^b	16^b	16^b	15^b

whole olives (WO, E = enzyme *Cytolase 0*; T = micronized food talc, mono unsaturated fatty acids (MUFA, stearic fatty acids (SFA. Data are means of three replicates (CVs≤7,1%). Within a row and each variety, values with different superscript letters are statistically different (*Tukey's* honestly significant difference range test, $p \leq 0.05$).

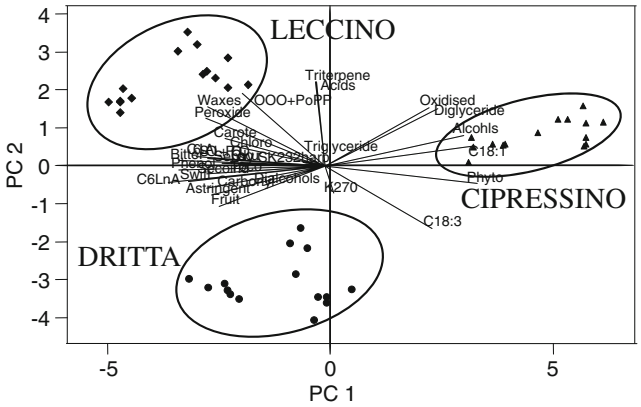

Fig. 1 Score and loading plot based on general analytical data by dimensions 1 and 2 from PCA. Oil samples group by olive variety. Statistical software used: SCAN®

Consequently, their green attributes appeared more pronounced with respect to the reference oils (Tables 1 and 2). They exhibited higher concentrations of biophenolics, *o*-diphenols, and ligstroside and other secoiridoid derivatives (noticeably oleuropein aglycons, i.e., dialdehydic forms of elenolic acid containing hydroxytyrosol) (Table 1). This phenomenon was most probably related to reduction of the thermal phenol oxidation processes, followed by quinonization and polymerization of the oxidized compounds. The lower phenol oxidation rate is likely also linked to the absence in DO paste of the kernel tissues, which was found to contain large amounts of polyphenoloxidases, peroxidases, and other oxidoreductase enzymes (Lavelli and Bondesan 2005). It can be pointed out that the *o*-diphenol polymers make up the brown catecholmelanin pigment that dissolves in the vegetation water and is largely responsible for the high polluting power of this liquid waste (Ranalli et al. 2002b).

The produced destoned oils displayed relatively higher contents of a-tocopherol, which is the most representative component of this bioactive minor fraction. Evidently, the soft preparation of olive paste is believed to be a critical step of the technological process safeguarding these and other valuable functional microcomponents (Table 1).

Related to higher levels of both green volatiles and minor polar compounds, these oils were scored by the sensory panel assessing the aroma and flavor as more marked and harmonic. The hedonistic properties, fragrance, and presumable consumer's acceptance and healthy value clearly discriminated from the reference oils (controls). The endurance to oxidation (induction time) was higher because of higher contents of natural phenol antioxidants (Table 1). On the contrary, the oxidative susceptibility (Cert et al. 1996) was not different from reference products (as the two oil kinds had similar contents of PUFA). Their potentially higher stability, as well as longer storability or shelf-life, was confirmed by their higher polyphenolics/PUFA ratio value (Table 1).

They exhibited lower contents of chloroplast pigments (Table 1), such as chlorophylls, pheophytins, and carotenoids (b-carotene, lutein, violaxanthin and neoxanthin). Such a phenomenon is most probably linked to lower effects of destoner with respect to metal crusher used in the reference extracting cycle on the fruit hypoderm tissue, where both chloroplasts and chromoplasts are essentially located. This hypothesis was corroborated by higher values of brightness (h%) and lower magnitudes of chroma (σ%) and integral color index (data not shown).

Other analytical parameters and indices referred to in Tables 1 and 2 were in general not significantly affected by the innovative extracting technology. The exogenous *Cytolase 0* enzyme complex, because of its biochemical actions on the vegetable tissues, enhanced the above mentioned effects of destoning on oil quality owing to further increased release of minor functional components from the vegetal tissues and incorporation into the oil phase. Such depolymerising enzyme system also increased the release of the fruit oil fraction, thus enhancing the oil outputs (1–2%), as confirmed by lower contents of unextracted oil in the byproducts (olive residue and wastewater). The processing aid of adding micronized food talc affected the oil output but not the quality level of products. The analytical features of the oils from DO-T were in general not different from the untreated destoned ones (DO). Also, DO-E did not differ from destoned oils treated with both enzyme and talc (DO-E+T).

By processing analytical data from monovarietal oils, statistical plots were obtained. The biplot obtained with the PCA is capable of discriminating between cultivars (Fig. 1). Along the first principle component (accounting for 72.5% of total variance) are discriminated Leccino (negative half) and Cipressino (positive half) oil samples, whereas along the second principle component (accounting for 15.8% of

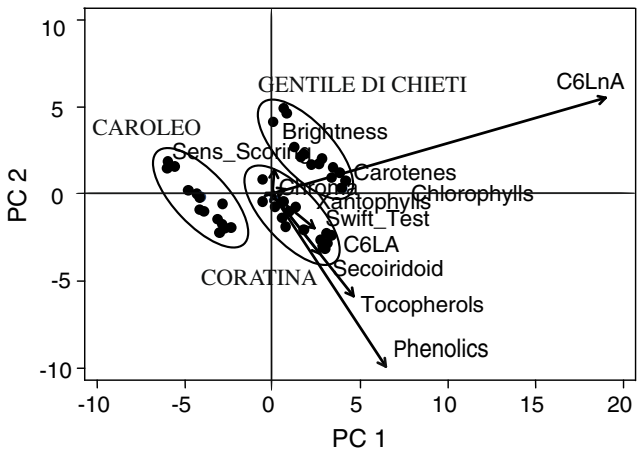

Fig. 2 Score and loading plot based on general analytical data by dimensions 1 and 2 from PCA. Oil samples tend to group by olive variety. Statistical software used: STATA®

total variance) Dritta oil samples are differentiated. Also, groupings of monovarietal oils appeared (Fig. 2).

Conclusions

The innovative olive processing technology, based on fruit destoning, enabled products of excellence that stood out for its delicate flavor and aroma, and nutritional valuable properties. In spite of the lower extracting yields, which can however be noticeably increased using effective biochemical (enzymes) and/or mineral (micronized food talc) processing aids, such a simplified extracting cycle could actually contribute to enhance the competitiveness of the olive oil sector. This will be possible thanks to both high-added values and high market prices of destoned oils. In the future, it is needed to gain further technological knowledge so as to minimize the meaningful losses of destoned oil in the byproducts (olive pomace and vegetation water).

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