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Effect of olive paste kneading process time on the overall quality of virgin olive oil

The influence of the olive paste malaxation time on the composition and the industrial output of oil was investigated. To this purpose, three Italian olive varieties (*Leccino, Dritta, Caroleo*) were processed with a centrifugal system for six malaxation periods (0, 15, 30, 45, 60 and 75 min). The concentrations of the majority of the oil constituents changed during the malaxation. However, these changes were not significant for all of them: the contents of β-carotene, the major xanthophylls, chlorophylls *a* and *b*, pheophytins *a* and *b* in the oils increased progressively with increasing malaxing times, whereas the contents of simple and hydrolysable phenols (secoiridoid derivatives), *o*-diphenols and total phenols decreased. A significant increase in total volatiles and green volatiles of the lipoxygenase cascade (C_6 aldehydes, C_6 alcohols, C_5 alcohols and C_5 carbonyls) was detected. An opposite trend was observed for the green C_6 esters. As a result, the global analytical quality, flavour, aroma and shelf-life of the oils were negatively affected. The oil yield increased substantially up to 45 min of paste malaxation times. Beyond 60 min, the yields tended to decrease.

Keywords: Malaxation time, olive oil, compositional quality, multivariate.

1 Introduction

Today, the biological and nutritional values of virgin olive oil and its tangible effects on human health are widely acknowledged. This commodity, which has been a staple delectable food in the Mediterranean area for thousand of years, has become more popular than ever in the United States, Canada, Australia, Japan, North Europe and other countries [1]. However, its properties can be improved by optimising the production techniques [2].

Paste malaxing (mixing), a basic step (following crushing) of the mechanical olive oil extraction process, was studied by several authors [3-8], but a comprehensive investigation of its effects on the oil composition has not been accomplished yet. Moreover, these studies were almost all carried out on a laboratory level and the available results are not univocal [5, 9, 10].

During this step, consisting of a slow and continuous kneading, many chemical-enzymatic processes and biosynthetic pathways take place. These significantly modify the oil's analytical features [3]. In addition, the lipoprotein membranes [10], which surround the oil droplets, are removed and re-formed repeatedly resulting in a mutual exchange of components between the oil and the water phase [3]. On the other hand, such membranes bind the minute oil droplets to the water droplets and the vegetable colloids (made up of hemicellulose, protein, pectin, *etc.*) thus form stable emulsions, which cannot be isolated or removed by mechanical means [3]. The emulsion is carried away with the by-products, such as olive pomace and vegetation water. The malaxation operation, inducing coalescence phenomena, causes the minute bound oil droplets to merge into large drops (diameter >30 µm), thus separating them from both colloids and water droplets [10]. In addition, this operation disrupts a proportion of the oily cells remaining uncrushed during the first step (grinding) allowing the recovery of another oil fraction. These phenomena can be increased by adding exogenous pectolytic enzymes to the olive paste at the start of the malaxation step [11].

In a previous work, we investigated the effects of the kneading temperature on the composition of oil fractions and on the extraction outputs [3]. This paper presents a thorough research on the effects on the oils quality and yield induced by kneading time. With these results we hope to provide oil-millers with substantial scientific guidelines to support the production of high-quality virgin olive oils in high yields.

2 Material and methods

2.1 Oil sample processing

Fresh, sound and healthy olives of three major Italian cultivars (*Leccino*, *Dritta* and *Caroleo*), produced on the farm of our Institute (Olive and Olive Oil Research Institute, Pescara, Italy) were used for the experiments. They were

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Tab. 1. Compositional features of the three processed olive varieties†.

Olive variety	Oil [%]	Moisture [%]	Solids [%]
Leccino	19.2	59.8	21.0
Dritta	23.1	52.6	24.3
Caroleo	21.6	49.8	28.6

† Data are means of three replicates (RSDs all <7%).

harvested by hand from October to November when their removal strength from the tree reached a value of 475-480 g. Their compositional features are given in Tab. 1. The cultivars had different rheological characteristics with *Leccino* cultivar being more difficult to process than *Dritta* and *Caroleo*.

For each variety and each malaxation time a homogeneous sample of 600 kg olives was processed, using an industrial continuous *Novoil EDJ/1* equipment (*Rapanelli Company*, Foligno, Italy). Each sample was divided into three 200 kg-parts, which were processed and tested as replicate batches. To grind the non-pitted, leafless and washed olive batches, a mobile *Inox* hammer crusher with a sieve size of 6 mm was used. The resulting oily pastes were kneaded in a mixer operated at 50 rpm for 0, 15, 30, 45, 60 and 75 min at the fixed and optimal temperature of 30 °C [3]. Furthermore, the oily pastes were centrifuged (600 kg $\times$ h^{-1}) at 3000 rpm for ~20 min with a two-phase automatic centrifugal decanter. During centrifugation, only the olive pastes from difficult olives (*Leccino variety*) were diluted with ~ 100 l $\times$ h^{-1} of lukewarm (30 °C) water.

The extracted oily must was separated into oil and water by means of a vertical automated discharge centrifuge operated at 9000 rpm. For each replicate a sample of olives (5 kg), oil (2 l), pomace (5 kg) and waste-water (liquid effluent) (1 l) were taken for analyses [11]. The relative contents of solids in olive fruits were determined as follows:

Solids% = 100-(oil% + moisture%)

All processing tests were performed carefully in the experimental oil mill under controlled conditions.

2.2 Oil sample analyses

Each oil samples was characterised by the determination of more than hundred analytical features. Both glyceridic and non-glyceridic components were evaluated. Individual triglycerides (expressed as % of total triglycerides) were determined by HPLC (High-Performance Liquid Chromatography), using a *Supelco supelcosil L-18* reversed-phase column (250 mm length, 45 mm i.d.; 5 μm particle size, *Supelco* Inc., Bellefonte Park, PA, USA). The mobile phase consisted of an acetone/acetonitrile (60/40, v/v) mixture, which was pumped at 1 ml min^{-1}. Samples were prepared to a concentration of 50 mg oil/1 ml acetone, and sample solutions were filtered through 0.5 μm filters. Injections (10 μl) were done manually [12].

Total triglycerides and total diglycerides (expressed as % of total glyceridic classes) were quantified by ^{13}C NMR (nuclear magnetic resonance). The oil sample (250 mg) was dissolved in deuterochloroform (0.5 ml) before running the spectra. Chemical shifts were relative to the signal of tetramethylsilane. The qualitative ratio 1,2 diglycerides/1,3 diglycerides was assessed [13].

Extraction and purification of phenolics were performed using a 30-g sample of dried virgin olive oil as described [3]. The phenolic extract was dissolved in acetone and derivatised with bis-trimethylsilyltrifluoroacetamide (BSTFA). The amounts of simple and hydrolysable phenols (secoiridoid derivatives) were expressed as mg/kg of resorcinol (internal standard). Total phenols and *o*-diphenols were determined colorimetrically at 725 and 450 nm, respectively, using *Folin-Ciocalteu* and *Arnow* reagents [11].

Volatiles with pleasant and unpleasant sensorial qualities were extracted from a sample of 50 g of oil in a 120 ml-*Drechsel* gas-washing bottle with a porous distributor. The volatiles fraction was stripped with nitrogen stream (1.2 dm^3/min; 37 °C) for 2 h, trapped on 50 mg of purified activated charcoal, and desorbed with 1 ml of diethyl ether. The thus processed fractions were analysed by a dynamic headspace (DHS)-high-resolution gas chromatography (HRGC) method, as described in *Ranalli* et al. [3]. The amounts of individual volatiles were expressed as mg/kg of nonan-1-ol (internal standard).

Oxidative stability (induction time of the peroxidising reactions) was evaluated using the automatic *Swift's* accelerated test carried out at 120 °C with an air flow rate of 20 l/h [14].

An analytical taste panel made up of eight assessors performed the quantitative descriptive sensory profiling (QDSP) at the Oil Quality Technology Department (OQTD) of our Institute, according to Annex XII of EC regulation n. 2568/91 [12]. Olfactory-gustatory-tactile evaluations were made and the results were reported on a standard profile sheet. The most remarkable sensory descriptors evaluated were fruity (green olives), cut green lawn, green leaf of twig, green olives, wild flowers, green banana, green tomato, almond, artichoke, apple, walnut husk, green hay, bitter and pungent [15]. Shades for each sensation were

also assessed. Each sensory attribute, including off-flavours, was evaluated on a six-point scale, with intensities ranging from 0 (no perception) to 5 (extreme). An overall evaluation of the magnitudes followed and the final sensory scoring was obtained applying a nine-point scale grading from 1 (lowest quality) to 9 (optimal quality). All oil samples were thermostated at 30 °C before sensory analysis.

Tocopherols were analysed by HPLC with a *M-porasil* direct-phase column (300 mm length, 3.9 mm i.d., 10 µm particle size from *Waters Corporation*, Milford, MA, USA), using a hexane/propan-2-ol (98.5:1.5, v/v) mixture as eluent and a UV (ultraviolet) detector at 292 nm wavelength (*Perkin-Elmer,* Norwalk, CT, USA) [14].

β-Carotene (pro-vitamin A) and major xanthophylls (lutein, violaxanthin and neoxanthin) were determined colorimetrically after separation by thin-layer chromatography (TLC), using *N,N*-dimethylformamide for the extraction and a mixture of petroleum ether 65-95C/acetone/diethylamine (10:4:1, v/v/v) as developer [16].

Fatty acids, waxes, long-chain aliphatic alcohols, higher triterpene alcohols, sterols and triterpene dialcohols, were determined by HRGC methods [14]. Free fatty acid content and peroxide index were determined by titrimetric methods [12]. Finally, the UV indices, chlorophylls *a* and *b* and pheophytins *a* and *b* concentrations, and chromatic parameters (chroma, brightness and hue) were determined by spectro-photometric methods [17].

The oil samples were freeze-stored until the moment of analysis. Suppliers as well as apparatus, reagents and solvents used for the above organic residual analyses have been reported in previous research papers dealing with virgin olive oil characterisation [18-20].

2.3 Univariate and multivariate statistical analyses

Trials according to a 6×3 factorial design (six olive paste kneading times x three olive varieties) were planned. All experiments were run in triplicates. The experimental methodology enabled the variance not attributable to the variety or kneading time to be removed. Based on this operating scheme, the experimental data were first processed by two-sided variance analysis (ANOVA); when a significant F value was found, means were separated using the *Spjotvoll* and *Stoline's* honest significant difference (HDS) *post hoc* pair wise test [21]. Multivariate techniques, such as principal component analysis (PCA), hierarchical cluster analysis (HCA), canonical discriminant analysis (CDA) and linear discriminant analysis (LDA) were also used [22-24]. The cross-validation procedure was used to determine the maximum number of significant components to avoid data over-fitting. The squared *Euclidean* distance and nearest neighbour method were used to obtain the dendrogram (tree graph). The statistical software packages *Minitab Release 13.1*® for Windows (*Minitab*, State College, PA, USA), *Statgraphics plus Professional Release 4.1*® for Windows (*Manugistic* Inc., Rockville, MD, USA), *Statistica Release 6.0*® *(Statsoft* Inc., Tulsa, OK, USA) and Excel Xp for Windows (*Microsoft Corporation,* Redmond, WA, USA) were used. A *Pentium IV* processor was used under Windows XP or Windows 98 second edition operating system.

3 Result and discussion

The time of olive paste malaxation did not seem to affect the saponifiable fraction (glycerides and fatty acids) of the obtained oils. Consequently, the qualitative ratio 1,2-diglycerides/1,3-diglycerides was not significantly modified. By contrast, several unsaponifiable components underwent variations in concentration resulting in modified qualitative levels of the oil. The peroxide index changes were not substantial which was probably related to the peroxide action against components with antioxidant activity (*e.g.*, polyphenols) [9]. Similar results were observed for the carbonyl index, UV absorption indices (K_{232}, K_{270} and ΔK) and free fatty acid content (notwithstanding the occurrence of endogenous lipase enzymes in the olive paste) [14].

Waxes, aliphatic long-chain alcohols, higher triterpene alcohols, triterpene dialcohols and steroids were released from the vegetable tissue to a relatively greater extent with the prolonging of the kneading operation. Consequently, they dissolved in slowly increasing amounts in the oil phase.

More significant variations in concentration were observed for pigments, phenolics and volatiles. Data concerning these components, which are major contributors to the sensory quality, and those concerning related important quality markers are given in Tabs. 2-4. Data concerning the unaffected or unsignificantly affected analytical parameters are omitted.

3.1 Chloroplast pigments, chromatic parameters and colour index

There are only few papers available in the literature concerning the pigment concentration changes induced by different olive paste kneading times [25]. We observed in all of the three virgin olive oil varieties examined an increase of concentration of either chlorophylls *a* and *b* or of pheophytins *a* and *b* (Mg-free chlorophyll derivatives) with increasing times of olive paste kneading, even though the pigment level appeared to be also dependent on the ge-

Tab. 2. Contents of chlorophylls, xanthophylls and carotenes and values of chromatic parameters in virgin olive oils from three varieties obtained after six different malaxing time periods [min]†.

Pigments and colour indices	*Leccino*						*Dritta*						*Caroleo*					
	0	15	30	45	60	75	0	15	30	45	60	75	0	15	30	45	60	75
Chlorophylls and pheophytins [mg kg^{-1}]	3.7^{a}	4.2^{b}	4.6^{b}	5.3^{c}	5.8^{d}	7.6^{e}	3.3^{a}	3.2^{a}	3.6^{b}	3.7^{b}	4.4^{c}	6.1^{d}	7.9^{a}	8.8^{b}	9.1^{c}	9.1^{c}	11.0^{d}	14.8^{e}
Xanthophylls [mg kg^{-1}]	3.26^{a}	3.54^{b}	3.50^{b}	3.83^{c}	3.94^{d}	4.22^{e}	3.10^{a}	3.07^{a}	3.34^{b}	3.37^{b}	3.68^{c}	4.00^{d}	3.50^{a}	3.71^{b}	3.68^{b}	3.87^{c}	4.25^{d}	4.62^{e}
Lutein [mg kg^{-1}]	2.78^{a}	2.98^{b}	2.95^{b}	3.10^{c}	3.15^{c}	3.26^{d}	2.54^{a}	2.50^{a}	2.68^{b}	2.68^{b}	2.85^{c}	3.09^{d}	2.88^{a}	2.99^{b}	2.97^{b}	3.09^{c}	3.29^{d}	3.42^{e}
Violaxanthin [mg kg^{-1}]	0.25^{a}	0.29^{a}	0.27^{a}	0.38^{b}	0.41^{d}	0.52^{e}	0.31^{a}	0.33^{a}	0.38^{b}	0.37^{b}	0.46^{c}	0.49^{c}	0.33^{a}	0.35^{ab}	0.39^{b}	0.43^{c}	0.53^{d}	0.66^{e}
Neoxanthin [mg kg^{-1}]	0.23^{a}	0.27^{b}	0.28^{b}	0.35^{c}	0.38^{c}	0.44^{d}	0.25^{ab}	0.24^{a}	0.28^{b}	0.32^{c}	0.37^{d}	0.42^{e}	0.29^{a}	0.33^{bc}	0.32^{b}	0.35^{c}	0.43^{d}	0.54^{e}
β-Carotene [mg kg^{-1}]	0.42^{a}	0.51^{b}	0.49^{b}	0.54^{c}	0.59^{d}	0.66^{e}	0.39^{a}	0.42^{b}	0.53^{c}	0.69^{d}	0.75^{e}	0.89^{f}	0.45^{ac}	0.51^{b}	0.47^{c}	0.59^{d}	0.71^{e}	0.98^{f}
Carotenoids [mg kg^{-1}]	3.68^{a}	4.05^{b}	3.99^{b}	4.37^{c}	4.53^{d}	4.88^{e}	3.49^{a}	3.49^{a}	3.87^{b}	4.06^{c}	4.43^{d}	4.89^{e}	3.95^{a}	4.22^{b}	4.15^{c}	4.46^{d}	4.96^{e}	5.60^{f}
Chroma [%]	59.4^{a}	62.9^{b}	64.5^{c}	66.5^{d}	69.4^{e}	75.2^{f}	65.0^{a}	65.1^{a}	65.8^{a}	66.9^{b}	68.2^{c}	73.5^{d}	84.9^{a}	86.3^{b}	87.3^{c}	87.5^{c}	88.6^{d}	88.7^{d}
Brightness [%]	82.0^{a}	81.5^{b}	80.5^{c}	78.1^{d}	76.7^{e}	65.1^{f}	84.4^{a}	83.5^{b}	79.9^{c}	78.7^{d}	78.3^{d}	77.5^{e}	76.7^{a}	71.6^{b}	69.5^{c}	68.1^{d}	60.8^{e}	59.6^{f}
Hue [nm]	576^{a}	576^{a}	576^{a}	577^{a}	577^{a}	576^{a}	576^{a}	577^{a}	576^{a}	577^{a}	576^{a}	577^{a}	577^{a}	578^{a}	577^{a}	577^{a}	577^{a}	577^{a}
Naudet's integral colour index	5.1^{a}	5.6^{b}	6.1^{c}	7.1^{d}	8.0^{e}	14.0^{f}	4.8^{a}	5.1^{b}	6.4^{c}	7.0^{d}	7.2^{d}	8.1^{e}	9.8^{a}	12.5^{b}	13.8^{c}	14.6^{d}	19.1^{e}	19.9^{f}

† Data are means of three replicates (RSDs all <10%). Within each variety, significant differences in the same row are shown by different superscript letters ($P \leq 0.05$) *(Spjotvoll* and *Stoline's* HSD test).

Tab. 3. Contents of phenols, resistance to autoxidation and sensory scoring in virgin olive oils from three varieties obtained after six different malaxing time periods [min]†.

Analytical oil parameters	*Leccino*						*Dritta*						*Caroleo*					
	0	15	30	45	60	75	0	15	30	45	60	75	0	15	30	45	60	75
Total phenols (as caffeic acid [mg kg^{-1}])	139^{a}	110^{b}	84^{c}	83^{c}	71^{d}	64^{e}	211^{a}	192^{b}	189^{b}	173^{c}	171^{c}	138^{d}	341^{a}	233^{b}	141^{c}	115^{d}	93^{e}	83^{e}
o-Diphenols (as caffeic acid [mg kg^{-1}])	68^{a}	59^{b}	45^{d}	48^{d}	42^{d}	32^{e}	127^{a}	106^{b}	102^{bc}	97^{c}	104^{c}	72^{d}	211^{a}	126^{b}	82^{c}	71^{d}	52^{e}	47^{e}
Tyrosol (free + aglycons, as resorcinol, [mg kg $^{-1}$])	19.5^{a}	17.3^{b}	15.0^{cd}	15.5^{c}	14.0^{d}	12.7^{e}	40.6^{a}	37.2^{b}	36.3^{b}	36.7^{b}	34.1^{c}	31.3^{d}	65.8^{a}	48.9^{b}	30.5^{c}	27.2^{d}	23.2^{e}	21.0^{f}
Hydroxytyrosol (free + aglycons, as resorcinol, [mg kg^{-1}])	25.0^{a}	22.1^{b}	19.7^{c}	19.1^{c}	17.9^{d}	15.1^{e}	68.1^{a}	59.5^{b}	58.7^{bc}	57.9^{c}	54.6^{d}	50.6^{e}	99.8^{a}	60.7^{b}	45.4^{c}	40.0^{d}	27.1^{e}	24.8^{f}
Sensory scoring (panel test)	7.3^{a}	7.1^{b}	7.0^{b}	7.0^{b}	6.8^{c}	6.7^{c}	7.7^{a}	7.6^{b}	7.6^{b}	7.5^{c}	7.5^{c}	7.3^{d}	8.0^{a}	7.8^{b}	7.4^{c}	7.3^{c}	7.3^{c}	7.1^{d}
Oxidative stability (*Swift's* [h])	10.9^{a}	9.9^{b}	9.1^{c}	9.3^{c}	8.8^{d}	8.3^{e}	12.9^{a}	12.8^{a}	12.0^{b}	11.0^{c}	10.8^{c}	10.4^{d}	15.4^{a}	13.4^{b}	13.1^{bc}	12.9^{c}	11.2^{d}	10.5^{e}

† Data are means of three replicates (RSDs all <10%). Within each variety, significant differences in the same row are shown by different superscript letters ($P \leq 0.05$) *(Spjotvoll* and *Stoline's* HSD test).

netic factor (olive variety, see Tab. 2). These increases, in general, were most intense at the kneading times of 60 and 75 min (Tab. 2) and were quite consistent with data reported in a previous work [25].

An analogous trend was found for the major xanthophylls, such as lutein, violaxanthin and neoxanthin, for β-carotene and total carotenoids (Tab. 2). This is due to a relationship between green and yellow lipochromes [16]. Evidently, these components, which are essentially located in the fruit hypoderm tissue [16], were freed to a greater extent when prolonging the oily paste kneading process, and in consequence greater amounts of them moved into the oil phase. However, there were simultaneously other phenomena influencing the chloroplast pigments during malaxation. So is the lipoxygenase (LOX) enzyme claimed to mediate the pigment destruction through the free radicals formed by its action on the polyunsaturated fatty acids with a *cis-cis* 1-4 pentadiene system [26].

As expected, the trend of chroma (saturation) and that of *Naudet's* integral colour index paralleled the pigment concentration, whereas the brightness (lightness) showed an opposite trend (being adversely related with the colour intensity) and that of hue was not related to the independent variable (kneading time) altogether (Tab. 2).

3.2 Simple and hydrolysable phenols (secoiridoid derivatives), sensory scoring, stability to autoxidation

With olive fruit disruption caused by the crushing operation, several enzyme species, triggering a number of biochemical pathways, become immediately active. Most of them exert their preponderant effect during the subsequent paste malaxation step, which is therefore primarily responsible for the profile changes in several analytical oil fractions [3]. The magnitudes of such biochemical reactions depend primarily on the enzyme levels characterising each olive cultivar (genetic store) and secondarily on exogenous factors. As a result, some new compounds not present originally in the olive drupe are generated [10]. Of prominent importance is the activity of β-glucosidase that could have a role in the production of phenol-aglycons (major secoiridoid derivatives having marked antioxidising properties) through hydrolysis of the oleuropein and demethyloleuropein glycosides [3].

Olive fruit is very rich in phenol constituents, which essentially include complex molecules (glycosides and esters), such as oleuropein, demethyloleuropein, ligstroside, verbascoside, flavonoids (flavonoid glycoside, luteolin-7-glucoside and rutin) and anthocyanins (cyanidin and delphinidin glycosides) [27]. From these large phenol

Tab. 4. Contents (as nonan-1-ol) of C_6 oxygenated compounds (green volatiles), from the LOX pathway, in virgin olive oils from three varieties obtained after six different malaxing time periods [min][†].

Volatiles [mg kg^{-1}]	*Leccino*						*Dritta*						*Caroleo*					
	0	15	30	45	60	75	0	15	30	45	60	75	0	15	30	45	60	75
Hexanal	5.1^{a}	15.8^{b}	31.4^{c}	32.4^{c}	48.4^{d}	61.3^{e}	4.2^{a}	15.2^{b}	37.2^{c}	43.6^{d}	55.2^{e}	67.8^{f}	8.0^{a}	8.8^{a}	15.2^{b}	20.9^{c}	30.5^{d}	41.9^{e}
Hexan-1-ol	8.3^{a}	9.5^{b}	9.3^{b}	10.8^{c}	11.5^{d}	13.9^{e}	7.8^{a}	8.4^{b}	9.1^{c}	10.3^{d}	11.7^{e}	13.0^{b}	6.9^{a}	7.1^{a}	7.0^{a}	7.8^{b}	8.9^{c}	10.4^{d}
Hexyl acetate	4.6^{a}	4.4^{a}	4.0^{ab}	3.6^{b}	3.0^{c}	2.0^{d}	4.9^{a}	4.4^{ab}	4.3^{b}	3.8^{c}	3.2^{d}	2.5^{e}	4.0^{a}	4.0^{a}	3.8^{ab}	3.3^{b}	2.9^{c}	2.0^{d}
Total amount C_6 compounds from LA	18.0^{a}	29.7^{b}	44.7^{c}	44.8^{c}	62.9^{d}	77.2^{e}	16.9^{a}	28.0^{b}	50.6^{c}	57.7^{d}	70.1^{e}	83.3^{f}	18.9^{a}	19.9^{a}	26.0^{b}	32.0^{c}	42.3^{d}	54.3^{e}
trans-2-Hexenal	427.1^{a}	444.5^{a}	473.9^{b}	488.4^{b}	518.7^{c}	548.9^{d}	431.2^{a}	444.4^{ab}	446.2^{b}	455.7^{b}	499.6^{c}	555.3^{d}	400.1^{a}	420.5^{b}	410.7^{ab}	441.2^{c}	496.8^{d}	530.5^{e}
trans-2-Hexen-1-ol	27.5^{a}	26.8^{a}	31.3^{b}	34.3^{c}	39.6^{d}	45.1^{e}	28.2^{a}	27.3^{a}	27.7^{a}	31.3^{b}	35.8^{c}	40.4^{d}	20.3^{a}	21.1^{a}	23.8^{b}	25.5^{c}	29.6^{d}	36.4^{e}
cis-3-Hexen-1-ol	7.8^{a}	7.0^{b}	8.4^{c}	9.0^{d}	10.2^{e}	12.5^{f}	7.0^{a}	7.9^{b}	7.7^{c}	8.3^{c}	9.8^{d}	12.5^{e}	6.1^{a}	6.6^{a}	7.3^{b}	8.4^{c}	9.6^{d}	11.5^{e}
cis-3-Hexenyl acetate	19.8^{a}	19.3^{a}	18.5^{b}	16.3^{c}	14.2^{d}	10.3^{e}	18.9^{a}	17.4^{b}	16.9^{b}	14.1^{c}	11.3^{d}	9.0^{e}	16.5^{a}	16.0^{ab}	15.5^{b}	14.4^{c}	13.2^{d}	10.5^{e}
Total amount C_6 compounds from LnA	482.2^{a}	497.4^{a}	532.1^{b}	548.0^{b}	582.7^{c}	616.8^{d}	485.3^{a}	497.0^{ab}	498.5^{b}	509.4^{b}	556.5^{c}	617.2^{d}	443.0^{a}	464.0^{b}	457.3^{b}	489.5^{c}	549.2^{d}	588.9^{e}
C_6 aldehydes/Σ C_6[‡]	86.2^{a}	87.3^{b}	87.6^{bc}	87.8^{c}	87.8^{c}	87.9^{c}	86.7^{a}	87.5^{b}	88.0^{c}	88.0^{c}	88.5^{d}	89.0^{e}	88.3^{ac}	88.7^{b}	88.1^{c}	88.6^{b}	89.1^{d}	89.0^{d}
C_6 alcohols/Σ C_6[‡]	8.7^{a}	8.2^{b}	8.5^{c}	9.1^{d}	9.5^{e}	10.3^{f}	8.6^{ac}	8.3^{b}	8.1^{b}	8.8^{c}	9.1^{d}	9.4^{e}	7.2^{a}	7.2^{a}	7.1^{a}	8.0^{b}	8.1^{b}	9.1^{c}
C_6 esters/Σ C_6[‡]	4.9^{a}	4.5^{b}	3.9^{c}	3.6^{d}	2.7^{e}	1.8^{f}	4.7^{a}	4.2^{b}	3.9^{c}	3.2^{d}	2.3^{e}	1.6^{f}	4.4^{a}	4.1^{b}	4.0^{b}	3.4^{c}	2.7^{d}	1.9^{e}

[†] Data are means of three replicates (RSDs all < 11%). Within each variety, significant differences in the same row are shown by different superscript letters ($P \leq 0.05$) (*Spjotvoll* and *Stoline's* HSD test).

[‡] Data are expressed in percentage in relation to the total C_6 compounds.

molecules, many simple or less complex phenol species, *i.e.* mainly phenyl acids and phenyl alcohols, are derived by chemical or enzymatic reactions [3] during the olive crushing and oily paste malaxing steps. However, the composition of the phenol fraction of olives and olive oil including its by-products has not yet been fully elucidated.

A major change involving the phenol fraction of olive fruit, essentially during crushing, is the formation of derivatives of *o*-diphenol compounds of the brown macromolecular catecholmelaninic pigment, through a combined quinonisation-polymerisation process mediated by polyphenoloxidase (PPO) enzymes. This pigment, which behaves as a polyacid, contains only small percentages of sugars and nitrogenous substances and, in contrary to other plant catecholmelanines, is fully watersoluble. It is therefore thoroughly washed away in the liquid effluent (waste-water) during the oil extraction process. It is a major waste-water component and is characterised by high specific COD (chemical oxygen demand) and BOD (biochemical oxygen demand), as well as by high resistance to microbial degradation [28].

In general, in our oils the concentration in total phenols and *o*-diphenols decreased as the olive paste kneading process time increased (Tab. 3). A similar trend was observed for the major free phenols (hydroxytyrosol and tyrosol) and the major hydrolysable phenols (hydroxytyrosol-aglycons and tyrosol-aglycons) (Tab. 3). The latter are secoiridoid-derived compounds identified as dialdehydic forms of elenolic acid [29, 30]. These trends are probably due to the oxidative reactions catalysed by oxidoreductase enzymes, such as polyphenoloxidase (PPO), peroxidase (POD) and LOX, which increase with increasing times of olive paste exposure to air. According to *Servili* et al. [31], PPO is almost completely inactivated during the crushing of olives and the only active oxidoreductase enzymes during oily paste malaxation are LOX and POD. LOX catalyses the formation of hydroperoxides and could be responsible for an indirect oxidation of secoiridoids. Studies are in progress to reduce the losses of oil phenol constituents during malaxation by treating the olive paste with either nitrogen flush or with antioxidants. The pH lowering of the paste could also be effective in this respect. The vegetable colloids could interfere positively in reducing the phenol oxidation phenomena [31].

Our findings are in general consistent with the results reported by other authors [9, 10, 32], including those reported by *Caponio* et al. [33] who found an increase in concentration for the phenol fraction 1 (including simple phenols) and a decrease in concentration for the phenol fraction 2 (including hydrolysable phenols), when prolonging the olive paste kneading process time. The last fraction, however, is quantitatively much more important, so the total phenol fraction concentration decreased.

Concerning the resistance to thermo-autoxidation of the produced oils, the trend of this parameter was parallel to that of the concentrations of phenol constituents (Tab. 3), thus confirming how these natural antioxidants are largely responsible for the oil shelf-life [10, 18, 27].

The sensory scoring assigned to the oils also showed a trend similar to that of phenols (Tab. 3) as these components are in addition related with the oil flavour characteristics, and noticeably with the levels of bitterness, pepperlike, astringency and fruitiness [34]. These descriptors decreased significantly and progressively in strength during malaxation. It is noteworthy that recently a cheaper and more reliable method based on the use of an electronic nose has been proposed for detecting virgin olive oil sensorial defects and predicting the shelf-life [35]. Also, several attempts have been made to correlate instrumental data to sensory ones with the aim to replace the costly panel test method [35].

Fig. 1 gives evidence that canonical discriminant analysis (CDA), based on the combined data of Tabs. 2 and 3, was effective in discriminating between oil varieties. In fact, the *Dritta* and *Caroleo* oils were discriminated along the first root (positive half; third and fourth quadrant, respectively), whereas the *Leccino* oils were discriminated along the second root (positive half; first quadrant). This means that the variance accounted for by the olive variety (genetic factor) overcame that explained by the technological factor examined (olive paste kneading time). These results were confirmed by the dendrogram (Fig. 2) obtained by cluster analysis (HCA) applied to the same data set used for the CDA analysis.

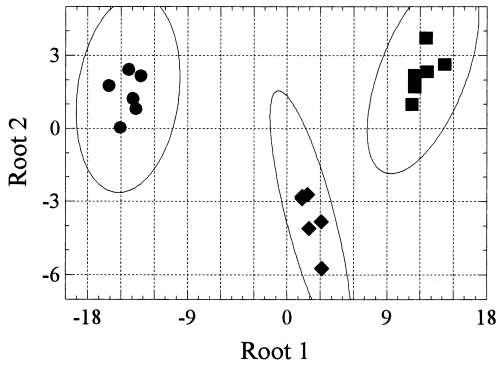

Fig. 1. Classification of *Leccino* (●), *Dritta* (◆) and *Caroleo* (■) oils, based on the data of Tabs. 1 and 2, using CDA. The genetic store effect prevails over that of olive paste malaxing carried out at six different time periods. Confidence ellipses are drafted at $P \leq 0.05$.

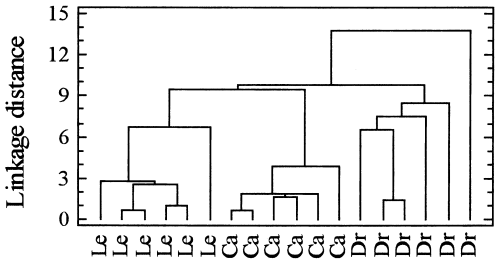

Fig. 2. Dendrogram, based on the data of Tabs. 1 and 2, showing the clustering of *Leccino* (Le), *Dritta* (Dr) and *Caroleo* (Ca) oils obtained by malaxing olive paste at six different time periods.

3.3 Profile of head-space volatiles

Volatiles are other key components relevant to quality and characteristic of virgin olive oil. Considerable changes in their composition take place during oily paste kneading (and olive crushing). These changes, besides depending on the kneading conditions (length of time and temperature level adopted), are strictly related to the olive variety processed (enzyme pattern). They are essentially of a biochemical nature. During malaxing even new volatiles can be biosynthesised, which then dissolve and accumulate in the oil phase [5, 10, 15].

Of major concern is the biogeneration of aliphatic C_6 and C_5 volatiles through the LOX pathways, as they are the main contributors to the green and fruity notes and the unripe fruit perceptions [10, 36, 37, 38]. The relationships amongst these volatiles (or other chemicals) and sensory descriptors have been established by chemometrics and the sensory wheel [39-41]. Also, data sets referring to these volatiles and sensory attributes have been successfully manipulated mathematically for the authentication of European virgin olive oils [42, 43]. Sensory wheels were in addition used for comparing QDA (quantitative descriptive analysis) panels [44].

The green metabolites have linolenic (LnA) and linoleic (LA) acids as their precursors, which in turn are formed by triglyceride hydrolysis mediated by acylhydrolase (AH). LOX transforms LnA and LA, characterised by a *cis-cis*-1,4-pentadiene structure, into their corresponding 9- and 13-hydroperoxides, in a ratio ranging between 65:35 and 55:45, respectively. Only the 13-hydroperoxides, from both LnA (13-HPOT) and LA (13-HPOD), are cleaved by hydroperoxide lyase (FAHL) into C_{12} oxo-acids, *cis*-3-hexenal and hexanal, as the enzyme has a high substrate specificity [36]. *Cis*-3-hexenal does not accumulate in the oil volatile fraction. Enzymatic transformation of the two aldehydes mediated by isomerases (IR), alcohol dehydrogenases (ADH) and alcohol acetyl transferases (AAT) yields the corresponding C_6 esters and C_6 alcohols. As a result, three branches of volatile C_6 metabolites are generated, two from LnA and one from LA [5, 37, 45, 46]. According to some latest researches, an additional branch of short-chain green volatiles, including oxygenated C_5 compounds, is biosynthesised through another LOX pathway [10]. In this case, 13-HPOT undergoes a β-scission yielding pentene dimers and pentenols through the alkoxyl radical. The subsequent oxidation of pentenols catalysed by an alcohol dehydrogenase yields C_5 carbonyl compounds [10].

The enzyme system of the LOX pathways [37] starts its activity as crushing begins, but seems to be mainly important during malaxation. Crushing, in fact, is a short step. The magnitude of such activity is genetically determined; however, it is significantly affected by exogenous factors. In particular, temperatures over 35 °C during malaxation can inhibit the hydroperoxide lyase and thus the LOX pathways [36].

In our oils, almost all the classes of oxygenated C_6 and C_5 volatiles (and thus the total green metabolites) increased in concentration with increasing times of olive paste kneading, even though in a few cases such increases were not statistically significant ($P \leq 0.05$) (Fig. 3). Only the amount of C6 esters decreased in the oils, likely due to a progressive inactivation of acyltransferase (Fig. 3). An analogous trend was noted for the ratios of total C_6 aldehydes, total C_6 alcohols and total C_6 esters to total C_6 volatiles (Tab. 4). However, the trends of individual volatiles were not always parallel to those of their corresponding groups. Our findings are in good agreement with those of literature [5, 10, 15], and partly also with those of the work [32] in which an increase in aldehydes and esters and a decrease in alcohols is reported for kneaded oils with respect to unkneaded ones (controls).

The C_6 aldehydes, among which the most abundant were the unsaturated (Tab. 4), were in general quantitatively more important (80.5%) compared to the C_6 alcohols (7.9%) and the C_6 esters (3.0%) (regardless of oil variety and time of paste malaxation) (Fig. 3). The relative amounts of C_5 carbonyls (4.6%) were comparable to those of C_5 alcohols (4.0%). The relative total amounts of C_6 volatiles (91.4%) were by far higher than the relative total amounts of C_5 volatiles (8.6%) (Fig. 3). The analytical data relating to individual C_5 compounds are not shown.

Total C_6 volatiles from linolenic acid (LnA) (92.4%) were largely predominant over total C_6 volatiles from linoleic acid (LA) (7.6%), because of highest values of *trans*-2-hexenal (82.8%) (Tab. 3). This was, among the agreeable volatiles ($C_6 + C_5$), the most abundant compound (75.8%).

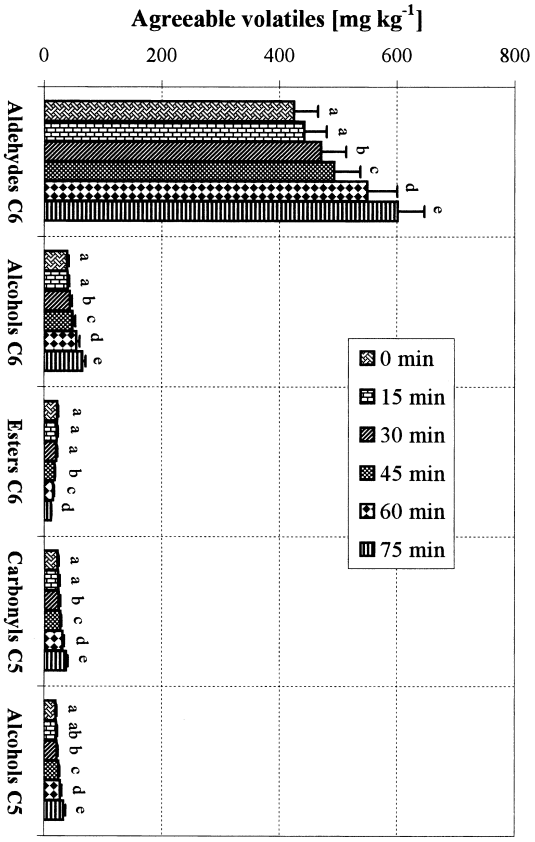

Fig. 3. Average content (expressed as nonan-1-ol) of C_6 aldehydes (a), C_6 alcohols (b), C_6 esters (c), C_5 carbonyls (d) and C_5 alcohols (e) arising from the LOX pathways in virgin olive oils (regardless of variety) obtained after six different malaxing time periods of olive paste. Within each class of volatiles, significant differences between means (n = 9) are shown by different letters ($P \leq 0.05$) (*Spjotvoll* and *Stoline's* HDS test). Error bars indicate standard deviation.

The major accumulation of volatile compounds originating from LnA is in agreement with the greater preference shown by ADH and AAT of olive fruits for unsaturated metabolites [37].

Fig. 4 shows how in all the three produced virgin olive oils varieties the total of undesirable volatiles accumulates significantly during olive paste kneading (more markedly at the times of 60 and 75 min). These undesirable volatiles included compounds from anaerobic fermentations of sugars (naturally occurring in the olive fruit), as well as branched aldehydes (2-methyl-butanol and 3-methyl-butanol) and branched alcohols from anaerobic degradation of amino acids (leucine, isoleucine and valine). They also included *n*-octane coming from hydroperoxide degradations. Some sugar degradation products (ethanol, acetic acid and ethyl acetate) are responsible at

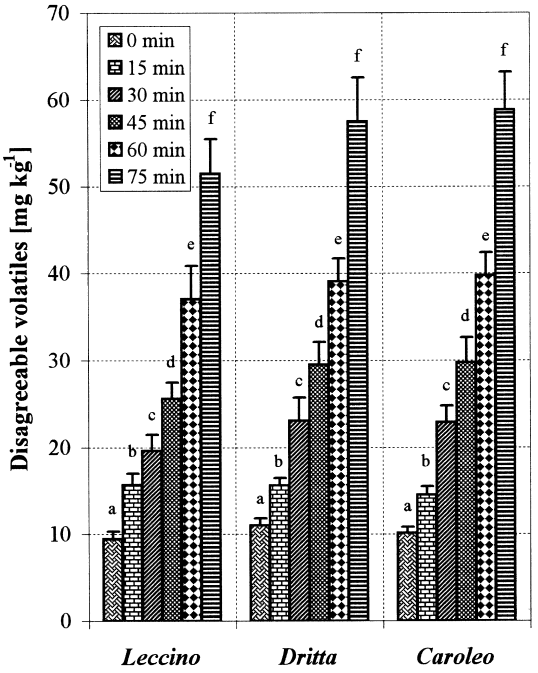

Fig. 4. Average content of total disagreeable volatiles (expressed as nonan-1-ol), from amino acid and sugar fermentations and hydroperoxide degradation, in three virgin olive oil varieties obtained after six different malaxing time periods. Within each variety, significant differences between means (n = 3) are shown by different letters ($P \leq 0.05$) (*Spjotvoll* and *Stoline's* HDS test). Error bars indicate standard deviation.

a certain concentration for the "winey" defect, whereas the branched alcohols (2-methyl-1-propanol, 2-methyl-butan-1-ol and 3-methyl-butan-1-ol) from amino acid degradation (as well as *n*-octane) relate with the "fusty" defect [3, 10]. However, the oil samples, even those obtained at the higher olive paste kneading times (60 and 75 min), were scored based on their attractive perceptions only, as they were free of any flaws.

Fig. 5 shows that canonical discriminant analysis (CDA), based on the data relating to green C_6 volatiles of the LOX cascade (Tab. 4), was effective in discriminating between oil varieties. In fact, the *Dritta* and *Leccino* oils were discriminated along the first root (positive half; third and fourth quadrant, respectively), whereas the *Caroleo* oils were discriminated along the second root (positive half; first quadrant). This means that the amount of variance accounted for by the genetic store of cultivar clearly prevailed over that explained by the time of olive paste malaxing.

Results (not shown) of other multivariate analyses (PCA, LDA) indicated how these techniques were also effective

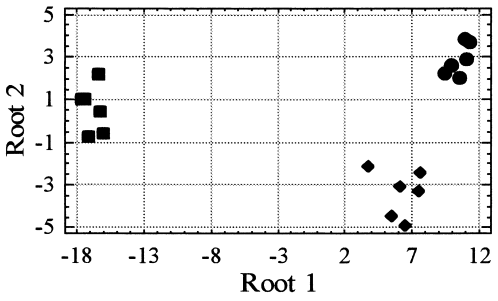

Fig. 5. Classification of *Leccino* (●), *Dritta* (◆) and *Caroleo* (■) oils, based on the data of Tab. 3, using CDA. The genetic store effect prevails over that of olive paste malaxing carried out at six different time periods.

in classifying the oil varieties correctly, proving again that the genetic factor accounted for a greater amount of variance than the technological factor studied (olive paste kneading process time).

3.4 Quantitative oil yields

The increases in oil yield (wt-% fruit oil basis), with any olive variety processed, were statistically significant ($P \leq 0.05$) up to an olive paste kneading time of 60 min. However, at this time the yield increases were no longer substantial. From 60-75 min the oil yields underwent a small decrease, likely due to a re-formation of oil-water or oil-solid emulsions. On average, regardless of the olive variety, the oil yields were 81.3, 82.1, 84.7, 87.8, 88.4 and 87.5% at the kneading times of 0, 15, 30, 45, 60 and 75 min, respectively.

These findings were substantiated by the lower total amount of residual oil (wt-%, olive fruit basis) found in the by-products, which was 3.71, 3.52, 3.30, 3.11, 2.99 and 3.05% at the kneading times of 0, 15, 30, 45, 60 and 75 min. The multiple comparisons between means (*Spjotvoll* and *Stoline's* HDS *post hoc* pair wise test) revealed that the differences were always statistically significant ($P \leq 0.05$), as were those concerning yields, but, equally, when the kneading time increased from 45-60 min the difference was no longer substantial. When the kneading time increased from 60 to 75 min, the by-products were characterised by an increase in total residual oil. Generally, these results fitted well with those of literature [6, 8, 25]

4 Conclusion

To sum up, there was evidence that the olive paste kneading time markedly influenced either the analytical characteristics of oil or the industrial outputs. This influence seemed to be even more meaningful than that ascribable to the crushing step [19]. The effect of kneading time on yields was positive but that exerted on the overall oil quality (notwithstanding the increase in total green volatiles) was not, because of progressive losses of natural phenol antioxidants (minor polar compounds), which are claimed to play a basic role in determining the sensory quality. This is a major component of the overall quality and will therefore determine the preference of the consumers. The significant losses of some green volatiles (esters) of the LOX cascade should also be stressed. To reduce such losses of key components and to achieve satisfactory yields at the same time, the operating conditions of malaxation should be a compromise considering both. Based on the results of the present research and on those achieved with a previous one [3], it can be suggested that the time and temperature of olive paste malaxation should not exceed the limits of 45 min and 30 °C, respectively. However, certain flexibility should be used in the practice, according to the olive paste rheology (olive variety) and the extraction system used. It should also be taken into account that the temperature parameter is the main factor characterising the beating process [5]; thus, longer kneading times might be adopted at lower paste temperatures.

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References

[1] *S. A. Wiseman, J. N. Mathot, N. J de Fouw, I. B. Tijburg:* Dietary nontocopherol antioxidants present in extravirgin olive oil increase the resistance of low density lipoproteins to oxidation in rabbits. Atherosclerosis **120** (1996) 15-23.

[2] *A. K. Kiritsakis:* Olive Oil Handbook. AOCS Press, Champaign (USA) 1998.

[3] *A. Ranalli, S. Contento, C. Schiavone, N. Simone:* Malaxing temperature affects volatile and phenol composition as well as other analytical features of virgin olive oil. Eur. J. Lipid Sci. Technol. **103** (2001) 228-238.

[4] *A. Koutsaftakis, F. Kotsifaki, E. Stefanoudaki:* Effect of extraction system, stage of ripeness, and kneading temperature on the sterol composition of virgin olive oil. J. Am. Oil Chem. Soc. **76** (1999) 1477-1481.

[5] *M. T. Morales, R. Aparicio:* Effect of extraction conditions on sensory quality of virgin olive oil. J. Am. Oil Chem. Soc. **76** (1999) 295-300.

[6] *R. Amirante, E. Cini, G. L. Montel, A. Pasqualone:* Influence of mixing and extraction parameters on virgin olive oil quality. Grasas y Aceites **52** (2001) 198-201.

[7] *G. Pieralisi, A. Ponzetti:* Technical and economical considerations on olive paste kneading as a function of the geometrical kneader form. Riv. Ital. Sos. Grasse **74** (1997) 73-77.

[8] *A. Lanzani, P. Bondioli, O. Cozzoli, L. Folegatti, E. Fedeli:* Influence of technical parameters on virgin olive oils quality in industrial practice. Note I. Riv. Ital. Sos. Grasse **67** (1990) 559-567.

[9] *G. Lercker, N. Frega, F. Bocci, M. Mozzon:* Volatile constituents and oxidative stability of virgin olive oils: influence of the kneading of olive paste. Grasas y Aceites **50** (1999) 26-29.

[10] *F. Angerosa, R. Mostallino, C. Basti, R. Vito:* Influence of malaxation temperature and time on the quality of virgin olive oils. Food Chem. **72** (2001) 19-28.

[11] *A. Ranalli, G. De Mattia:* Characterization of olive oil produced with a new enzyme processing aid. J. Am. Oil Chem. Soc. **74** (1997) 1105-1113.

[12] *European Union Commission:* Regulation EC 2568/91 on the characteristics of olive and olive-pomace oils and on their analytical methods. European Community's Official Gazette l **248** (1991) 1-36.

[13] *G. Vlahov:* Regiospecific analysis of natural mixtures of triglycerides using quantitative ^{13}C nuclear magnetic resonance of acyl chain carbonyl carbons. Mag. Res. Chem. **36** (1998) 359-362.

[14] *A. Ranalli, M. L. Ferrante, G. De Mattia, N. Costantini:* Analytical evaluation of virgin olive oil of first and second extraction. J. Agric. Food Chem. **47** (1999) 417-424.

[15] *M. T. Morales, F. Angerosa, R. Aparicio:* Effect of the extraction conditions of virgin olive oil on the lipoxygenase cascade: chemical and sensory implications. Grasas y Aceites **50** (1999) 114-121.

[16] *A. Ranalli, P. Cabras, E. Iannucci, S. Contento:* Lipochromes, vitamins, aromas and other components of virgin olive oil are affected by processing technology. Food Chem. **73** (2001) 445-451.

[17] *A. Ranalli, A. Tombesi, M. L. Ferrante, G. De Mattia:* Respiratory rate of olive drupes during their ripening cycle and quality of oil extracted. J. Sci. Food Agric. **77** (1998) 359-367.

[18] *A. Ranalli, A. Malfatti, P. Cabras:* Composition and quality of pressed virgin olive oil extracted with a new enzyme processing aid. J. Food Sci. **66** (2001) 592-603.

[19] *A. Ranalli, G. De Mattia, M. L. Ferrante:* The characteristics of percolation olive oils produced with a new enzyme processing aid. Int. J. Food Sci. Technol. **32** (1998) 247-258.

[20] *A. Ranalli, G. Modesti, M. Patumi, G. Fontanazza:* The compositional quality and sensory properties of virgin olive oil from a new olive cultivar – I-77. Food Chem. **69** (2000) 37-46.

[21] *J. C. Hsu:* Multiple comparisons, theory and methods. Chapman & Hall (USA) 1996.

[22] *Minitab software package:* User's Guide, Release 13.1, Minitab, State College, PA, USA (1995) 25-39.

[23] *Statistica software package:* User's Guide, Release 6.0, Statsoft, Tulsa, OK, USA (1999) 77-102.

[24] *Statgraphics Plus software package:* User's Guide, Release 4.1, Manugistics, Rockville, Maryland, USA (1998) 9-53.

[25] *L. Di Giovacchino:* Olive oil extraction by direct centrifugation of olive pastes. Note I: Influence of the kneading process. Riv. Ital. Sos. Grasse **68** (1991) 413-420.

[26] *M. Jarén-Galán, C. Carmon-Ramón, M. I. Minguez-Mosquera:* Interaction between chloroplast pigments and lipoxygenase enzymatics extract of olives. J. Agric. Food Chem. **47** (1999) 2671-2677.

[27] *M. Servili, M. Baldioli, R. Selvaggini, E. Miniati, A. Macchioni, G. F. Montedoro:* High-performance liquid chromatography evaluation of phenols in olive fruit, virgin olive oil, vegetation waters, and pomace and 1D- and 2D-nuclear magnetic resonance characterization. J. Am. Oil Chem. Soc. **76** (1999) 873-882.

[28] *A. Ranalli:* Microbiological treatment of oil-mill waste water. Grasas y Aceites **43** (1992) 16-19.

[29] G. *F. Montedoro, M. Servili, R. Selvaggini, R. Miniati, E. Macchioni:* Simple and hydrolyzable phenolic compounds in virgin olive oil. Spectroscopic characterization of the secoiridoid derivatives. J. Agric. Food Chem. **41**(1993) 2228-2234.

[30] *F. Angerosa, N. D'Alessandro, F. Corana, G. Mellerio:* Characterization of phenolic and secoiridoid aglycons present in virgin olive oil by gas chromatography–chemical ionization mass spectrometry. J. Chrom. **48** (1996) 81-90.

[31] *M. Servili, M. Baldioli, G. F. Montedoro:* The mechanisms influencing the concentration in polyphenols of virgin olive oil. Abstract, presented at the "Olive oil quality" Congress, Florence (Italy), 1992.

[32] *G. F. Montedoro, L. Garofolo:* The qualitative characteristics of virgin olive oils. The influence of variables such as variety, environment, preservation, extraction, conditioning of the finished product. Riv. Ital. Sos. Grasse **61** (1984), pp. 157-168.

[33] *F. Caponio, V. Alloggio, T. Gomes:* Phenolic compounds of virgin olive oil: influence of paste preparation techniques. Food Chem. **64** (1999), pp. 203-209.

[34] *A. K. Kiritsakis:* Flavor components of olive oil–a review. J. Am. Oil Chem. Soc. **75** (1998) 673-681.

[35] *R. Aparicio, S. M. Rocha, I. Delgadillo, M. T. Morales:* Detection of rancid defect in virgin olive oil by the electronic nose. J. Agric. Food Chem. **48** (2000) 853-860.

[36] *J. J. Salas, J. Sánchez:* The decrease of virgin olive oil flavour produced by high malaxation temperature is due to inactivation of hydroperoxide lyase. J. Agric. Food Chem. **47** (1999) 809-812.

[37] *J. M. Olías, A. G. Pérez, J. J. Ríos, L. C. Sanz:* Aroma of virgin olive oil: biogenesis of the "green" odor notes. J. Agric. Food Chem. **41** (1993) 2368-2373.

[38] *R. Aparicio, M. T. Morales:* Characterisation of olive ripeness by green aroma compounds of virgin olive oil. J. Agric. Food Chem. **46** (1998) 116-1192.

[39] *M. T. Morales, V. Alonso, J. J. Ríos, R. Aparicio:* Virgin olive oil aroma: relationship between volatile compounds and sensory attributes by chemometrics. J. Agric. Food Chem. **43** (1995) 2925-2931.

[40] *R. Aparicio, M. T. Morales, V. Alonso:* Relationship between volatile compounds and sensory attributes of olive oils by the sensory wheel. J. Am. Oil Chem. Soc. **73** (1996) 1253-1264.

[41] *F. Gutiérrez, I. Varona, M. A. Albi:* Relation of acidity and sensory quality with sterol content of olive oil from stored fruit. J. Agric. Food Chem. **48** (2000) 1106-1110.

[42] *R. Aparicio, M. T. Morales, V. Alonso:* Authentication of European virgin olive oils by their chemical compounds, sensory attributes, and consumers' attitudes. J. Agric Food Chem. **45** (1997) 1076-1083.

[43] *R. Aparicio, J. J. Calvente, M. T. Morales:* Sensory authentication of European extra-virgin olive oil varieties by mathematical procedures. J. Sci. Food Agric. **72** (1996) 435-457.

[44] *R. Aparicio, M. T. Morales:* Sensory wheels: a statistical technique for comparing QDA panels–application to virgin olive oil. J. Sci. Food Agric. **67** (1995) 247-257.

[45] *A. G. H. Hatanaka, T. Kajjwara, J. Sekiya:* Biosynthetic pathway for C_6 aldehydes formation from linolenic acid in green leaves. Chem. Phys. Lipids **44** (1987) 341-361.

[46] *H. W. Gardner:* Recent investigation into lipoxygenase pathway of plants. Biochim. Biophys. Acta **1084** (1991) 221-239.

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