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Lipochromes, vitamins, aromas and other components of virgin olive oil are affected by processing technology

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Abstract

A stone mill-integral decanter (SD) olive processing line was compared with a traditional stone mill-press (SP) line, a discontinuous method, that, if properly used, yields high-quality virgin olive oils. With "difficult olives" (not easy to process), the SD line yielded oils characterised by: (1) higher contents of pleasant volatiles, tocopherols, β-carotene, xanthophylls, chlorophylls, pheophytins and waxes; (2) higher values of the 1,2-diglycerides/1,3-diglycerides ratio, integral colour index and chroma; (3) lower contents of phenols, secoiridoids, unpleasant volatiles, aliphatic alcohols, triterpene alcohols and sterols; and (4) lower values of turbidity, brightness and oxidative stability. With "non-difficult olives" (easy to process), whose paste needed to be fluidised with lukewarm water, the SD line yielded oils showing higher contents of pleasant volatiles and tocopherols, lower contents of unpleasant volatiles and comparable contents of phenols and lipochromes. The content of organophosphorus pesticide residues did not depend on the processing method.

Keywords: Lipochromes; Vitamins; Aromas; Virgin olive oil; Processing; Chemometrics

1. Introduction

Virgin olive oil, a typical product of the Mediterranean area, is one of the oldest vegetable oils and the only one that is consumed in its crude form (unrefined). It is valued for its unique aroma and flavour, and for its long shelf-life. Today, its nutritional and healthful effects are universally recognised (Boskou, 1996).

Processing is a major factor affecting the virgin olive oil quality. Pressed oil obtained under the proper processing conditions is generally of excellent quality (Kiritsakis, 1998). In a previous investigation, we observed that virgin olive oils extracted by a centrifugal integral decanter (also called dual-phase decanter) were inferior to the homologous pressed oils (Ranalli & Ferrante, 1998). These results suggested that the phenomenon could be due to the crusher used by the two processing methods: a stone mill is used by the discontinuous pressing method, while metal crushers (hammer, disk and roller types) are used by the continuous centrifugal method. Such metal crushers cause a significant increase of the paste temperature, which results in a negative effect on the oil quality (Ranalli, Ferrante, De Mattia & Costantini, 1999).

In this work, we tested an innovative continuous processing line (SD) made up of a stone mill (instead of the usual metal crusher) and a dual-phase decanter, which was compared with a stone mill-press (SP) processing line. The analytical patterns of the two resulting oil kinds were elucidated. The goal of this research was to obtain a centrifuged virgin olive oil of highest quality (comparable or superior to the pressed virgin olive oil), in order to replace the discontinuous pressing step with the continuous centrifuging one. To our knowledge, no investigation has been carried out on this important topic. The results of this research should lead to meaningful technological advances in virgin olive oil production.

2. Materials and methods

2.1. Olive varieties processed

The experiments were carried out by processing under proper conditions (Ranalli, De Mattia, & Ferrante, 1997), hand-picked fresh olives (*Olea europaea* L.) from three selected Italian cultivars (*Dritta, Leccino* and

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Cassanese), which were characterised by good sanitary state and normal ripeness. The olive trees were 30-year-old and had been given drop irrigation and fertirrigation from the time of full bloom to fruit maturation (April–November).

The *Dritta* olives were higher in oil content (19.3%) than *Leccino* olives (18.7%) and *Cassanese* olives (13.5%). The first variety was easy to process, whereas the last two were difficult as their moisture content was very high (>60%).

The oil from *Dritta* cultivar, called "Aprutino Pescarese", received a European POD (protect origin denomination) quality trademark. The *Leccino* and *Dritta* oils represent a significant proportion of the bottled oil trade in Italy and in Europe. The *Cassanese* cultivar is widespread in the Calabrian region and produces relatively big-size olives that are also used for direct consumption as table olives (Ranalli & Modesti, 1999).

2.2. Preparation of olive batches, processing, sampling

For each olive variety, a homogeneous sample of 2.4 t was processed, 1.2 t by a stone mill-integral decanter line and 1.2 t by a stone mill-press line. Each half was divided into four equal 300-kg parts which were processed and tested as replicate batches. A centrifugal processing equipment (Rapanelli, Foligno, Italy) and a pressing one (Pieralisi, Jesi, Italy) were used. The processing conditions were the same as outlined in a previous work (Ranalli & Ferrante, 1998). No water was added to the olive paste prior to pressing. The centrifugal method differed from the pressing one since the extraction step was not carried out by hydraulic superpresses but by a horizontal two-phase centrifuge (decanter). For the grinding operation, a stone mill was used with both processing methods. The kneaded "difficult olive pastes" were centrifuged (600 kg h^{-1}) at 3500×***g*** by adding 200 l h^{-1} of tap water (heated to 30°C), whereas no processing water was added with "non-difficult olive pastes". For each test, samples of olives (raw material), oil, olive pomace and waste water were drawn for analyses.

2.3. Oil sample analyses

β-carotene (pro-vitamin A) and other major yellow lipochromes (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 65–95°C: acetone: diethylamine (10:4:1, v/v) as developer (Mínguez-Mosquera, Gandul Rojas & Gallardo-Guerrero, 1992; Ranalli, 1992).

Tocopherols (vitamin E) were evaluated by high-pressure liquid chromatography (HPLC) with a direct-phase M-porasil column (3.9×300 mm, 10 μm, from Water Corporation, Milford, MA, USA), using a hexane-propan-2-ol (98.5:1.5, v/v) eluent (flow rate 1.3 ml min^{-1}, pressure 600 psi) and a UV detector at 292 nm wavelength (Ranalli & Ferrante, 1998).

Pleasant and unpleasant volatiles were stripped from the oil samples with N_2 (1.2 l min^{-1}, 37°C for 2 h), trapped on 50 mg of activated charcoal and eluted with 1 ml of diethyl ether. Next, they were simultaneously analysed by a dynamic head-space (DHS)-high-resolution gas chromatography (HRGC) method (Ranalli, Ferrante, De Mattia, & Costantini, 1999), using a Mega Series 5160 gas chromatograph (Carlo Erba, Milan, Italy) fitted with a silica carbowax 20-M capillary column (50 m length, 0.32 mm i.d., 0.5 μm film thickness) (Nordion Ltd., Helsinki, Finland) and equipped with an on-column injection system, a CO2 cryogenic accessory (to hold the oven temperature at 25°C) and a flame ionisation detector (FID). The oven temperature program was as follows: isotherm at 25°C for 7 min, from 25 to 33°C at 0.8°C min^{-1}, from 33 to 80°C at 2.4°C min^{-1}, from 80 to 155°C at 3.7°C min^{-1}, final isotherm at 155°C for 20 min. The temperature of the detector was held at 240°C. The carrier gas was H_2 at 30 kPa. The injection volume was 0.5 μl. Quantitation was achieved by peak area integration with a Mega Series integrator (Carlo Erba, Milan, Italy). The internal standard was nonan-1-ol (>99% pure) that was directly added (7–8 mg) to the oil sample (50 g).

The residues of organophosphorus pesticides, such as dimethoate, fenthion and fenthion sulfoxide (main metabolite of fenthion), which had been used to control the attacks of olive fly, *Bactocera (Dacus) oleae*, the key insect pest in the Mediterranean area, were also quantified (Cabras, Angioni, Melis, Minelli & Pirisi, 1997). Two grams of olive oil were weighed in a 30-ml screw-capped tube; 2 ml of hexane were added and, after agitation, another 10 ml of acetonitrile. The tube was agitated in a rotatory shaker for 30 min. The acetonitrile layer was allowed to separate; next, 7.5 ml were poured into a 10-ml beaker and allowed to evaporate to dryness under a nitrogen stream. The residue was taken up with 1.5 ml of hexane containing the internal standard and injected for GC analysis. The pesticide recovery was more than 100% for dimethoate and around 98% for fenthion. Pesticide analytical standards were purchased from Ehrenstorfer (Augsburg, Germany). Triphenylphosphate (>99% pure) was used as the internal standard and was of analytical grade (Janssen, Geel, Belgium). An HRGC Mega 5160 gas chromatograph (Carlo Erba, Milan, Italy), fitted with an NPD-40 nitrogen-phosphorus detector, an AS 550 autosampler (Carlo Erba, Milan, Italy), a split-splitless injector and a Durabond fused silica column (30 m length, 0.25 mm i.d., 0.25 μm film thikness, from J&W Scientific, Folsom, CA, USA) with DB 1701 (14% cyanopropyl-phenyl-methylpolysiloxane) liquid-phase was employed.

The other analytical parameters assessed (including the green lipochromes) are listed in Tables 1 and 2. The

Table 1
Analytical and compositional characteristics of virgin oils obtained from three olive varieties processed by a stone mill-integral decanter (SD) line compared to a stone mill-press (SP) line[a]

Analytical oil parameters	*Dritta*		*Leccino*		*Cassanese*	
	SD	SP	SD	SP	SD	SP
Lutein (mg kg^{-1})	3.01 (0.13)	2.88 (0.13)	2.75 (0.09)*	2.31 (0.08)	3.76 (0.23)*	3.25 (0.15)
β-Carotene (mg kg^{-1})	0.91 (0.03)	0.80 (0.03)	0.86 (0.05)*	0.65 (0.02)	1.29 (0.06)*	1.03 (0.04)
Violaxanthin (mg kg)	0.53 (0.02)	0.48 (0.02)	0.45 (0.02)*	0.32 (0.02)	0.65 (0.04)*	0.51 (0.02)
Neoxanthin (mg kg^{-1})	0.50 (0.02)	0.44 (0.03)	0.42 (0.01)*	0.30 (0.01)	0.63 (0.02)*	0.48 (0.02)
Chlorophylls and pheophytins (mg kg^{-1})	7.5 (0.4)	6.3 (0.3)	5.6 (0.4)*	4.0 (0.2)	10.5 (0.5)*	8.8 (0.4)
Chroma (%)	62.2 (4.0)	57.3 (3.3)	54.1 (3.3)*	48.3 (2.3)	64.8 (2.5)*	58.6 (2.7)
Brightness (%)	78.1 (3.7)	80.1 (4.6)	83.6 (4.9)*	87.3 (3.8)	76.2 (2.1)*	79.5 (3.1)
Total phenols (as caffeic acid, mg kg^{-1})[b]	140 (9)	145 (6)	88 (4)**	105 (4)	151 (7)**	176 (8)
Hydroxytyrosol (free + aglycones) (as resorcinol, mg kg^{-1})[c]	39 (1)	44 (2)	22 (1)*	35 (2)	52 (3)**	75 (3)
Tyrosol (free + aglycones) (as resorcinol, mg kg^{-1})[c]	20 (1)	22 (1)	9 (1)*	15 (1)	21 (1)**	30 (1)
Carbonyl index	2.7 (0.1)	2.5 (0.1)	3.6 (0.2)	3.6 (0.2)	4.5 (0.2)	4.1 (0.2)
Peroxide value (meq O_2 kg^{-1})	6 (0.2)	5 (0.2)	5 (0.3)	5 (0.3)	6 (0.2)	5 (0.2)
K_{232}[d]	1.41 (0.07)	1.43(0.09)	1.36 (0.08)	1.37 (0.07)	1.28 (0.05)	1.32 (0.06)
K_{270}[d]	0.10 (0.00)	0.12 (0.01)	0.08 (0.00)	0.10 (0.01)	0.09 (0.00)	0.11 (0.01)
Free acitity degree (as oleic acid, g kg^{-1})	3.3 (0.2)	3.5 (0.2)	3.1 (0.2)	3.3 (0.2)	2.1 (0.1)	2.0 (0.1)
Panel test (score)	7.3 (0.2)	7.2 (0.2)	7.1 (0.2)*	6.7 (0.2)	7.4 (0.2)*	7.8 (0.2)
Turbidity (NTU)[e]	250 (12)**	570 (27)	153 (7)**	310 (14)	62 (3)*	88 (4)

[a] Data are means of four replicates. Standard errors are shown in brackets. Within a row, each SD value with one or two asterisks is significantly different from the corresponding SP value (Tukey's HSD range test; $*P \leqslant 0.05$; $**P \leqslant 0.01$).
[b] As determined by colorimetric method.
[c] As determined by HRGC method.
[d] Specific extinction.
[e] Nephelometric turbidity units.

Table 2
Other analytical and compositional characteristics of virgin oils obtained from three olive varieties processed by a stone mill-integral decanter (SD) line compared to a stone mill-press (SP) line[a]

Analytical oil parameters	*Dritta*		*Leccino*		*Cassanese*	
	SD	SP	SD	SP	SD	SP
α-Tocopherol (mg kg^{-1})	88.3 (3.7)*	74.5 (3.4)	125.2 (7.9)*	112.6 (5.4)	105.8 (4.7)*	91.0 (5.1)
γ-Tocopherol (mg kg^{-1})	0.3 (0.0)**	0.1 (0.0)	0.2 (0.0)*	0.1 (0.0)	0.2 (0.0)*	0.1 (0.0)
Pleasant volatiles (as nonan-1-ol, mg kg^{-1})	426 (26)**	356 (17)	502 (33)*	440 (19)	420 (25)*	363 (18)
Oxidative stability (Swift's test, h)	9.3 (0.4)	9.6 (0.4)	7.6 (0.5)*	8.9 (0.4)	13.1 (0.6)*	14.1 (0.7)
1,2-Diacylglycerols/1,3-diacylglycerols ratio	1.7 (0.1)*	1.2 (0.0)	1.4 (0.1)*	1.0 (0.0)	1.8 (0.1)*	1.4 (0.1)
Total waxes (C_{34}-C_{46}, mg kg^{-1})	291 (14)*	276 (13)	305 (15)**	279 (15)	245 (9)**	211 (9)
Total β-sitosterol (mg kg^{-1})[b]	1200 (74)*	1324 (67)	1389 (69)*	1470 (65)	1445 (86)	1449 (72)
δ^5-Avenasterol (mg kg^{-1})	157 (6)*	208 (10)	160 (7)	165 (8)	95 (5)*	125 (7)
Aliphatic alcohols (mg kg^{-1})	129 (5)*	175 (8)	173 (11)*	221 (10)	154 (7)*	173 (9)
Triterpene alcohols (mg kg^{-1})	1519 (88)**	1705 (83)	1153 (74)*	1225 (59)	684 (29)*	825 (40)
Phytol (mg kg^{-1})	16.5 (0.7)*	18.7 (0.8)	21.5 (1.0)*	24.6 (1.1)	14.6 (0.7)**	19.4 (0.9)
Citrostadienol (mg kg^{-1})	8.5 (0.4)*	9.7 (0.6)	11.6 (0.7)*	14.7 (0.8)	9.1 (0.4)*	12.0 (0.7)
Geranylgeraniol (mg kg^{-1})	3.6 (0.2)	4.5 (0.2)	5.5 (0.3)*	6.7 (0.3)	3.7 (0.2)*	4.8 (0.3)
Triterpene dialcohols (mg kg^{-1})	2.1 (0.1)	2.4 (0.1)	1.7 (0.1)	1.5 (0.1)	1.8 (0.1)	2.0 (0.1)
Saturated fatty acids (%)[c]	14.7 (0.4)	14.4 (0.5)	14.4 (0.6)	14.4 (0.5)	12.1 (0.4)	12.8 (0.5)
Monounsaturated fatty acids (%)[c]	77.8 (2.2)	77.9 (2.9)	80.4 (2.5)	80.3 (2.5)	80.3 (2.2)	80.3 (1.8)
Polyunsaturated fatty acids (%)[c]	7.5 (0.2)	7.7 (0.2)	5.2 (0.1)	5.3 (0.1)	7.6 (0.2)*	6.9 (0.2)

[a] Data are means of four replicates. Standard errors are shown in brackets. Within a row, each SD value with one or two asterisks is significantly different from the corresponding SP value (Tukey's HSD range test; $*P \leqslant 0.05$; $**P \leqslant 0.01$).
[b] Data are the sum of Δ^5-23-stigmastadienol + clerosterol + β-sitosterol + sitostanol + Δ^5-avenasterol + Δ^5-24-stigmastadienol.
[c] Data are expressed in percentage in relation to the total fatty acids.

HRGC, HPLC, ^{13}C nuclear magnetic resonance (NMR) and other analytical methodologies used for oil (and by-product) sample analyses have been outlined in previous reports (Ranalli & Angerosa, 1996; Ranalli, Sgaramella & Surricchio, 1999), where chemicals, solvents, apparatus and suppliers have also been given. The oil samples were stored frozen (at $-20^{\circ}C$) until analysed.

2.4. Statistical analyses

A 2×3 factorial design (two processing methods× three olive varieties) was adopted. Average data concerning both yields and analytical variables were statistically tested with the two-sided variance analysis (ANOVA) with replications. If the null hypothesis was rejected, Tukey's honestly significant difference (HSD) test was applied to separate the means (Hsu, 1996). Probabilities higher than $P=0.05$ were considered non-significant. Data concerning analytical oil and waste water variables were also processed by principal component analysis (PCA) and hierarchical cluster analysis (HCA; Meloun, Militki & Forina, 1992). The cross-validation procedure was used to determine the maximum number of significant components to avoid data over-fitting. The statistical software packages Statistica (Statsoft Inc., Tulsa, OK, USA) and Scan for Windows (Minitab Inc., State College, PA, USA) were used. A Pentium III processor was used under Windows 98 2nd edition operating system. A 486 processor was also used under Windows 95 operating system.

3. Results and discussion

3.1. Carotenoids, chlorophylls, chromatic parameters, colour index

The quality of virgin olive oil is closely associated with the colour, since many of the pigments in the oil occur in concentrations that reflect the oil quality. Many of these components undergo degradation during olive ripening and oil storage and their loss, as well as the increase in their breakdown products, indicates the level of oil freshness (Ranalli & Modesti, 1999).

The contents of β-carotene, major xanthophylls (lutein, violaxanthin and neoxanthin), chlorophylls and pheophytins, tended to be higher in the centrifuged oils (Table 1), suggesting that the centrifuging process (compared to the pressed one) could result in a greater effect on the vegetable hypoderm tissue, where these substances are essentially located (Ranalli, 1992). However, the lipochrome concentration was not statistically different ($P\leqslant 0.05$) with "non-difficult olives" (when no water was added to the oily paste). It is probable that with "difficult olives" the increased polarity due to the addition of processing water induced more of the non-polar carotenoids and chlorophylls to partition into the oil. There was a linear relationship ($r=0.9833$***) between the values of total carotenoids and total chlorophylls.

The values of chroma (Table 1) and integral colour index (not shown) recorded for the produced oils were consistent with those of the lipochromes. The colour parameters (brightness excepted) generally show higher values when lipochrome concentration increases (Ranalli et al., 1997).

3.2. Tocopherols, phenols, oxidative stability

α-Tocopherol accounted for more than 98% of the tocopherol fraction (which comes primarily from the fruit seed; Kiritsakis, 1998). The centrifuged oils contained more α- and γ-tocopherol even when water was added to the oily paste (Table 2). All oil samples contained minute amounts of β- and δ-tocopherol (<0.05 mg kg^{-1}).

The average values of total phenols, *o*-diphenols, tyrosol (free + aglycones) and hydroxytyrosol (free + aglycones; Servili, Baldioli, Selvaggini, Macchioni & Montedoro, 1999), for the oils obtained by the two processing methods from "non-difficult olives", were not statistically different (Table 1). Only when water was added for processing "difficult olives" did the centrifuged oils have a lower content of phenols (Table 1). Phenolics are soluble in both oil and water (more in water) and therefore a portion of these substances was carried away by the processing water, due to modification of the partition equilibrium between the two non-mixable liquid phases (Ranalli & Angerosa, 1996).

The *o*-diphenol components, which accounted for around 60% of the phenol fraction, are largely responsible for the shelf-life of virgin olive oil because of their antioxidising potency (Baldioli, Servili, Perretti & Montedoro, 1996). Consequently, these substances affected the oxidative stability parameter. On the other hand, the tocopherol fraction did not seem to exert visible antioxidising effects, α-tocopherol having essentially vitamin properties (whereas a significant antioxidising action is exerted by δ-, γ- and β-tocopherol) (Psomiadou & Tsimidou, 1998). In fact, the oxidative stability of the centrifuged *Dritta* oil, that had (with respect to the homologous pressed one) higher contents of tocopherols and similar contents of phenols, was not higher. Also, the centrifuged *Leccino* and *Cassanese* oils, that were higher in tocopherols but lower in phenols, showed a significantly ($P\leqslant 0.05$) lower oxidative stability (Table 2).

3.3. Head-space volatile components and sensory scoring

All the centrifuged oils contained greater amounts of pleasant volatiles (Table 2). The differences, however, were less significant when these oils were obtained from olive pastes with added processing water. In this case, in

fact, some water-soluble volatiles (e.g. alcohols) were carried away (Ranalli & Angerosa, 1996).

The most significant volatiles were the C_6 compounds (aldeydes, alcohols and esters) from the lipoxygenase (LOX) pathway (Table 3), which are largely responsible for the green notes of virgin olive oil (Morales & Aparicio, 1999). These compounds form, essentially, during crushing and malaxing (operations causing damage to the fruit cells) from the polyunsaturated fatty acids containing a *cis*-*cis*-1,4-pentadiene structure (Aparicio & Morales, 1998). *Trans*-2-hexenal was by far the most representative volatile among those of the LOX cascade (Table 3). This compound is characterised by a pleasant odour of fresh cut grass as well as by a bitter taste (Morales & Aparicio, 1999).

The centrifuged oils showed a higher ratio of C_6 esters (and frequently of C_6 aldeydes) to total C_6 compounds and a lower ratio of C_6 alcohols to total C_6 compounds (Table 3). Reasonable amounts of C_5 alcohols (e.g. 1-penten-3-ol and 2-penten-3-ol) and C_5 carbonyl compounds (e.g. pentan-3-one and pent-1-en-3-one) were also present in the oils. These compounds are further contributors to the green aroma and could represent another group of volatiles arising from the LOX pathway (Morales & Aparicio, 1999).

By contrast, the pressed oils were richer in unpleasant volatiles (*n*-octane, ethyl acetate, ethanol, isobutyl alcohol, *n*-amyl alcohol and acetic acid; data not shown), whatever the olive variety processed, probably due to the discontinuous extraction that might result in fermentation processes involving the pulp particles remaining on the filtering diaphragms (Ranalli, Ferrante, De Mattia & Costantini, 1999). However, both centrifuged and pressed oils were free of organoleptic defects; therefore they were scored by only evaluating their positive sensorial attributes.

Both pleasant volatiles and phenols strongly affect the flavour and fruitiness of virgin olive oil (Ranalli, Sgaramella & Surricchio, 1999). The balance between green and fruit notes produces the fragrant flavour (Aparicio & Morales, 1998; Morales & Aparicio, 1999). The centrifuged oils frequently received higher sensory scores and their overall acceptability was greater, whereas their bitterness index did not differ significantly from the pressed oils. In fact, the employment of a stone mill (instead of the usual metal crusher), in the continuous extraction cycle, led to centrifuged oils having no marked bitterness, sharp or astringent notes.

A good positive linear relationship between the bitter and pungent attributes was found ($r = 0.9648$**). This result was expected since both sensations are generated in the same gustative papillae. In particular, bitter sensation is mainly due to an interaction between polar molecules (secoiridoids) and the lipid portion of taste papillae membrane, and pungent perception to the stimulation from polar molecules of the trigeminal free endings associated with taste buds in fungiform papillae (Withehead, 1985).

Table 3
C_6 volatiles from lipoxygenase (LOX) pathway determined in virgin olive oils obtained from three olive varieties processed by a stone mill-integral decanter (SD) line compared to a stone mill-press (SP) line[a]

C_6 volatiles	*Dritta*		*Leccino*		*Cassanese*	
	SD	SP	SD	SP	SD	SP
Hexanal	27.0 (1.3)	29.4 (1.3)	15.1 (0.6)	15.4 (0.8)	28.4 (1.3)**	11.2 (0.4)
Hexan-1-ol	29.3 (1.0)*	25.7 (1.0)	30.6 (1.3)**	19.9 (0.5)	28.8 (1.1)**	16.2 (0.8)
Hexyl acetate	4.5 (0.2)**	2.1 (0.2)	6.6 (0.3)**	3.4 (0.1)	8.4 (0.4)*	5.2 (0.2)
Total amount C_6 compounds from LA[b]	60.8 (2.1)*	57.2 (2.1)	52.3 (1.8)**	38.7 (1.5)	65.6 (3.1)**	32.6 (1.4)
Trans-2-hexenal	327.2 (15.2)**	233.1 (2.4)	397 (17.5)**	295.0 (8.4)	238.4 (11.8)*	201.9 (7.6)
Trans-2-hexen-1-ol	56.3 (2.9)*	48.9 (2.3)	95.0 (4.0)**	64.4 (2.5)	32.0 (1.4)	30.5 (1.4)
Cis-3-hexen-1-ol	9.7 (0.5)	8.2 (0.3)	3.0 (0.1)	4.9 (0.2)	54.6 (2.0)*	49.3 (1.9)
Cis-3-hexenil-acetate	4.5 (0.2)**	2.1 (0.1)	6.3 (0.2)*	4.2 (0.2)	7.8 (0.4)*	5.4 (0.3)
Total amount C_6 compounds from LnA[c]	397.7 (13.1)**	292.3 (13.9)	501.7 (21.6)**	368.3 (13.8)	332.8 (17.1)**	287.1 (13.9)
C_6 aldeydes/ΣC_6[d]	77.2 (3.3)**	75.1 (3.5)	74.5 (2.7)*	76.2 (7.6)	67.0 (2.8)*	66.6 (3.1)
C_6 alcohols/ΣC_6[d]	20.8 (1.0)**	23.7 (0.9)	23.2 (1.1)*	21.9 (1.1)	29.0 (1.3)*	30.0 (1.4)
C_6 esters/ΣC_6[d]	2.0 (0.1)*	1.2 (0.1)	2.3 (0.1)*	1.9 (0.1)	4.1 (0.2)*	3.3 (0.2)

[a] Data are expressed as mg kg^{-1} of nonan-1-ol (internal standard) and are means of four replicates. Standard errors are shown in brackets. Within a row, each SD value with one or two asterisks is significantly different from the corresponding SP value (Tukey's HSD range test).

[b] LA, lineoleic acid.

[c] LnA, linolemic acid.

[d] Data are expressed in percentage in relation to the total C_5 compounds. $^*P \leqslant 0.05$.

$^{**}P \leqslant 0.01$.

3.4. Other compositional features

The values of turbidity were steadily higher in the pressed oils (Table 1), probably due to greater contents of minute solid particles. The analytical variables (UV specific absorptions, free acid content, peroxide index, carbonyl index, triterpene dialcohol content, triglyceride and diglyceride percentage and fatty acid composition) were not modified by the processing method. Only the qualitative 1,2-diglycerides/1,3-diglycerides ratio (Howart & Vlahov, 1996) was relatively higher in the centrifuged oils (Table 2), possibly due to the centrifugation extraction that could decrease the transformation (isomerisation) rate of 1,2-diglycerides to 1,3-diglycerides (Table 2).

The contents of sterols and superior aliphatic and triterpene alcohols tended to be substantially higher in the pressed oils (Table 2). Evidently, the pressing extraction resulted in a greater release of these compounds. Finally, the content of waxes was higher in the centrifuged oils (Table 2), likely due to the added processing water that might alter the wax partition coefficients.

3.5. Pesticide residues

Qualities of virgin olive oil and other crop commodities are primarily defined by their sanitary, toxicological and safety features (Kiritsakis, 1998). Most of the oil samples contained no detectable (<0.001 mg kg^{-1}) pesticide residues. Only the *Dritta* samples contained dimethoate residues that exceeded the limit set (for refined olive oil) by the Codex Alimentarius Commission of the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO, 1996). However, with the *Dritta* cultivar, the last treatment with dimethoate was improperly carried out just a week before harvest. Fenthion and fenthion sulfoxide residues were present only in the *Leccino* samples; however they did not exceed the maximum limit (1 mg kg^{-1} for virgin olive oil) set by FAO/WHO Codex Alimentarius. Fenthion and dimethoate are the most common organophosphorus insecticides used in the olive-growing countries (Cabras et al., 1997).

3.6. Oil yields

The SD processing line gave average oil yields (% w/w, fruit oil basis) equal to 88.5, 87.3 and 87.0% with the *Dritta*, *Leccino* and *Cassanese* olive varieties, respectively. The corresponding yields of the SP line were 88.8, 88.3 and 87.9%. The differences were not substantially significant even with "difficult olives", when moderate amounts of oily pulp particles were carried away by the added processing water (Ranalli & Angerosa, 1996). These results were confirmed by the lower oil content found in the by-products. It is noteworthy that all the waste waters produced were characterised by a high content of natural phenols, which was up to 40-fold higher than that recorded for the oils.

3.7. Multivariate analysis results

Three dimensions of the PCA model, based on the major analytical oil parameters (Tables 1 and 2), were found to be significant and explained 84% of variance. The PCA scores of samples on the 1 and 2 dimensions are given in Fig. 1. The two PCA factors were effective in discriminating between oil varieties. The *Dritta* oil was discriminated along the second component (positive half), while the *Leccino* and *Cassanese* oils were differentiated along the first component (negative and positive side, respectively). The genetic factor effect, as expected, predominated over the processing method effect. This was confirmed by HCA. In fact, the dendrogram (Fig. 2) showed three blocks, each consisting of one variety, with a similarity percentage $>50\%$ (*Dritta* and *Leccino* oils) and $<50\%$ (*Cassanese* oil).

Among the main analytical waste water variables (Ranalli, Sgaramella & Surricchio, 1999), three dimensions of the PCA model were found to be significant and

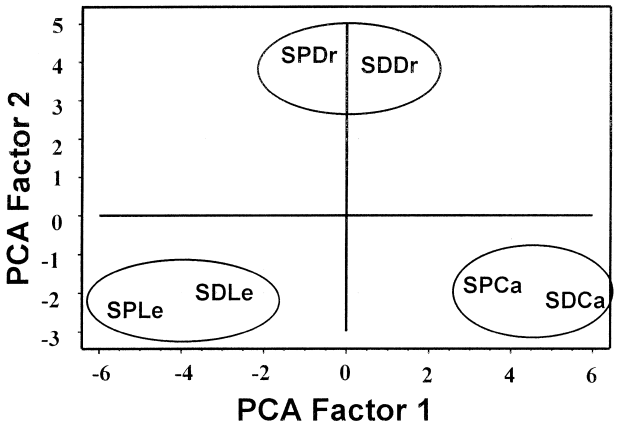

Fig. 1. Score plot, by dimensions 1 and 2 from principal component analysis (PCA), of the centrifuged (SD) and pressed (SP) virgin olive oils from *Dritta* (Dr), *Leccino* (Le) and *Cassanese* (Ca) olive varieties.

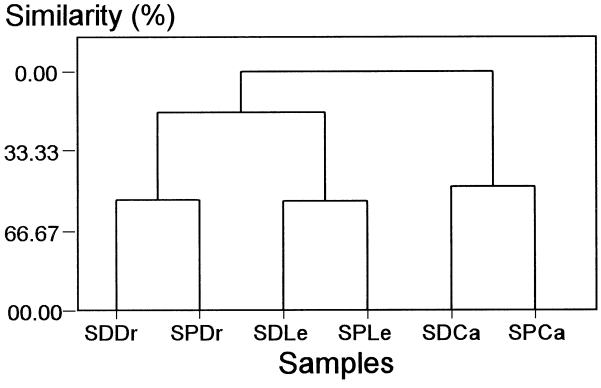

Fig. 2. Dendrogram showing the clustering of the *Dritta*, *Leccino* and *Cassanese* oil samples. Sample abbreviations are explained in Fig. 1 legend.

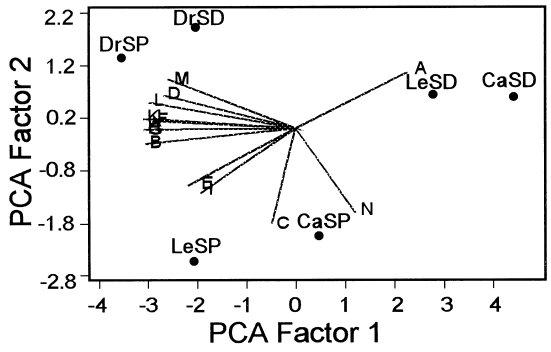

Fig. 3. Biplot, by dimensions 1 and 2 from principal component analysis (PCA), for the centrifuged (SD) and pressed (SP) waste waters from *Dritta* (Dr), *Leccino* (Le) and *Cassanese* (Ca) olive varieties.

explained 98% of the total variance. However, the first two dimensions alone explained a large percentage of variance (88%). The PCA scores of samples on the 1 and 2 dimensions (Fig. 3) and the dendrogram (not shown) indicated that the waste water samples arising from "difficult olives" were discriminated by the processing method, while the samples from "non-difficult olives" were discriminated by the olive variety.

4. Conclusion

The SD processing line, with "non-difficult olives" (*Dritta* variety), yielded (with respect to the SP processing line) virgin olive oils exhibiting a higher qualitative standard. This better quality was substantially ascribable to higher contents of pleasant volatiles and tocopherols (vitamin E) and to lower contents of unpleasant volatiles. With "difficult olives" (*Leccino* and *Cassanese* varieties), the centrifuged oils showed a lower content of phenolics, but their overall quality was higher (because of higher contents of vitamins, aromas and lipochromes). Thus, the innovative SD processing line might gradually replace the classic SP line in the good olive-growing areas, in order to produce extra-virgin olive oils of excellent quality. The SD line also increased the processing speed (as the extraction step became continuous); consequently the olive storage time (a factor which relates negatively to quality) decreased.

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