Analytical Evaluation of Virgin Olive Oil of First and Second Extraction

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Virgin olive oils from percolation (first extraction) have been compared with the corresponding oils from centrifugation (second extraction). The former were characterized by (i) higher contents of total phenols, *o*-diphenols, hydroxytyrosol, tyrosol-aglycons, tocopherols, *trans*-2-hexenal, total volatiles, and waxes; (ii) higher values of resistance to autoxidation and of turbidity; (iii) higher sensory scores; (iv) higher ratios of campesterol/stigmasterol, *trans*-2-hexenal/hexanal, and *trans*-2-hexenal/total volatiles; (v) lower contents of chlorophylls, pheophytins, sterols, and aliphatic and triterpene alcohols; (vi) lower alcoholic index and color indices; (vii) similar values of acidity, peroxide index, and UV (ultraviolet) spectrophotometric indices; (viii) similar percentages of saturated and unsaturated fatty acids, triglycerides, and diglycerides; and (ix) similar values of glyceridic indices. Stigmastadienes, *trans*-oleic, *trans*-linoleic, and *trans*-linolenic acid isomers were not detected in the two genuine oil kinds. Hence, the qualitative level of the first extraction oil was superior to the second extraction one.

Keywords: *Olive processing; percolation−centrifugation technology; quality and genuineness characteristics of products; shelf life; flavor; yields*

INTRODUCTION

Virgin olive oil is a foodstuff rich in oleic acid (74−78%) and contains an amount of linoleic acid (7.2−7.6%) similar to that in human milk (Kannel et al., 1979). Oleic acid is more resistant to the peroxidizing cell process compared to the polyunsaturated fatty acids and gives rise to a reduced formation of free radicals (Baudet et al., 1986). This fatty acid is also in part responsible for the increase of the plasma HDL (high-density lipoprotein) cholesterol and apoprotein A_1 and for the decrease of LDL (low-density lipoprotein) cholesterol and apoprotein B (Grundy, 1986). For this reason it plays a role in the prevention of the cardiovascular diseases (arteriosclerosis, myocardium infarct, cerebral ictus, etc.), which are the main causes of mortality in the industrialized countries (Kannel et al., 1979; Baudet et al., 1986; Grundy, 1986; Esteva et al., 1986). Oleic acid also reduces thrombogenesis, VII hemostatis factor, and blood platelet aggregation, contributes to the stabilization of arterial pressure and glycaemia (hematic insulin level), and even stimulates the growth of bones (Guezmir, 1977).

Virgin olive oil is also rich in natural antioxidants (phenols and tocopherols) and in aromatic compounds (Ranalli et al., 1997), as it is extracted from the drupe (a fruit where such substances are plentiful) by using only mechanical systems and not chemical treatments. The latter are not allowed by law (Ranalli and De Mattia, 1997). Therefore, virgin olive oil (which also is very digestible) is claimed to be the best vegetable oil for human health (Mattson and Grundy, 1985). Hence, commercial frauds involving virgin olive oil, and particularly its adulteration with inferior value vegetable oils, are very frequent (Goodacre et al., 1992, 1993).

Both the traditional discontinuous pressing cycle and the continuous centrifugation and percolation−centrifugation systems are in use to process olives (Ranalli and Serraiocco, 1996a,b). Olive crushing, kneading of the resulting paste, oil must extraction, and separation of must into oil and water are all steps common to the three processing systems. Product (oil), solid byproduct (husk), and liquid byproduct (vegetable water) are obtained in those processes (Ranalli and Angerosa, 1996).

The various operations of the olive processing cycle use purpose-developed machines, which differ according to the processing system (Ranalli, 1992). In the traditional cycle, mill-stone and hydraulic presses are used, respectively, for milling and pressing of olives. In the continuous cycles, metal crushers (hammer, disk, and roller types) are used to grind olives, and a horizontal centrifugal decanter ("De Sludger" or "Flottweg" type) is used for the centrifugation of olive paste (Ranalli and De Mattia, 1997). A vertical centrifuge (for separation of the oily must into oil and water) and an olive washing machine (which also separates the leaves from olives) are used by all three processing systems (Ranalli, 1992).

The first percolation extractor version (1911) was called "Acapulco", the second version (1930) was called "Acapulco-Quintanilla", and the third version (1951), which was manufactured in Spain by Buendía, was called "Alfin"; today the latter is manufactured in Italy, where it is called "Sinolea", and is a special surface tension extractor made up of 6000 steel blades (Ranalli et al., 1997).

An in-depth analytical comparative study, concerning the oils from three olive varieties processed by the two types of extractors making up the percolation−centrifugation system (and one increasingly applied in the olive-

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Table 1. Compositional Characteristics and Ripening Degree of the Three Processed Olive Varieties[a]

olive variety	oil (%)	moisture (%)	solids (%)	ripening degree
Leccino	19.1	45.3	35.6	4.4
Coratina	22.2	45.8	32.0	1.7
Dritta	24.0	50.2	25.8	3.1

[a] Means of six replicates (CVs all <8%).

producing countries), has been accomplished, and the results are reported in this paper. The two oil kinds are both important regarding percent, even though the percentages are influenced by the olive variety processed.

MATERIALS AND METHODS

Olive Processing. Three olive varieties (*Leccino*, *Coratina*, and *Dritta*), produced on the farm of our institute ("Istituto Sperimentale per l'Elaiotecnica", Pescara, Italy), were processed using an industrial scale apparatus. The oil mill, a Novoil ED J/1 machine (Rapanelli firm, Foligno, Italy), consisted of a percolation extractor ("Sinolea") and a centrifugal decanter. Double extraction tests were performed. Two kinds of oil were obtained: (i) percolation oil (first extraction) and (ii) centrifugation oil (second extraction).

The steps of the industrial process were as follows: (i) washing and defoliation of olive lots; (ii) milling of nonpitted drupes by a mobile hammer crusher; (iii) kneading of the resulting paste for 1 h at 30 °C (the plant was equipped with two kneaders which enabled a continuous processing); (iv) first extraction of oily must from paste by "Sinolea" (duration 50 min); (v) second extraction by an innovative centrifugal decanter equipped with special head plates; and (vi) separation of the oily must into oil and water by means of an automated discharge centrifuge.

During the second extraction, the paste (550 kg h^{-1}) was fluidized by adding 400 L h^{-1} of tap water at 30 °C. For each variety a homogeneous sample of 1.8 ton of olives was divided into six equal 300 kg parts which were processed and tested as replicate batches.

During testing, samples of the olives were taken as well as byproducts and oils by applying the same sampling techniques briefly described in previous reports (Ranalli, 1992; Ranalli and Serraiocco, 1996a). The methods applied to perform the analytical assessments on the olives, husk, and vegetable water (olive juice) are also summarized in these papers.

Compositional Characteristics and Ripening Degree of the Olive Varieties Processed. From each replicate batch for all varieties, a 5 kg olive sample was withdrawn at random, from which subsamples were taken for the measurements. The maturation index of the three olive varieties was determined according to the method developed by the "Estación de Olivicultura y Elaiotecnia", Mengibar, Jaén, Spain (Uceda and Frías, 1975). The pigmentation extent of the epicarp and mesocarp of the drupes was evaluated by using a measuring scale based on seven pigmentation levels. For each determination, a sample of 100 olives was used, and percentages of oil, moisture, and solids of the fruits were also determined. The mean values (of six replicates) are given in Table 1; all have variation coefficient (CV) < 8%. The ripening degree of drupes is one of the main factors affecting the oil quality. The relative content of fruit liquids and solids affects the rheological characteristics of the paste and therefore the ability of the machines to process the paste.

Oil Sample Analyses. Volatiles were analyzed by HRGC (high-resolution gas chromatography) (Ranalli and Angerosa, 1996) using a carbowax 20 M capillary column (25 m, 0.32 mm i.d., 0.20 μm film thickness, from Nordion Ltd., Helsinki, Finland). The volatiles were extracted by stripping at 37 °C in a nitrogen stream, and entrapping by activated charcoal; they were then eluted with diethyl ether. The internal standard was nonan-1-ol (>99% pure). The chromatogram (aromagram) of this fraction showed many peaks but, as yet, only 21 components have been identified (Ranalli and Angerosa, 1996), such as *n*-octane, ethyl acetate, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, ethanol, 3-pentanone, 1-penten-3-one, hexanal, isobutyl alcohol, *trans*-2-pentenal, 1-penten-3-ol, isoamyl alcohol, *trans*-2-hexenal, *n*-amyl alcohol, 2-penten-1-ol, 1-hexanol, *cis*-3-hexen-1-ol, *trans*-2 hexenol, acetic acid, 1-octanol, and 2-butanone.

Tocopherols were analyzed by HPLC (high-performance liquid chromatography) (Ranalli and De Mattia, 1997) with a direct-phase M-porasil column (3.9 m × 300 mm, 10 μm, from Waters Corporation, Milford, MA), using a hexane-propan-2-ol (98.5:1.5, v/v) eluent and a UV detector at 292 nm wavelength. α-, β-, γ-, and δ-tocopherol were detected.

Phenolics were analyzed by HRGC (Angerosa et al., 1995), using a capillary column (25 m, 0.32 μm i.d., 0.1 μm film thickness, from Lab. Service Analitica Ltd., Anzola Emilia, BO, Italy). After extraction with methanol, the resulting extract was concentrated to dryness by a rotary evaporator and dissolved in 10 mL of acetonitrile. This solution, after three washings with hexane, was evaporated in a vacuum at <35 °C. The residue was dissolved in acetone, and to 1 mL of this solution was added 150 μL of BSTFA (bis-trimethylsilyltrifluoroacetamide). After 1 h the prepared mixture was injected (0.5 μL) into a gas chromatograph. The internal standard was resorcinol (>99% pure). Free tyrosol and hidroxytyrosol and tyrosol- and hydroxytyrosol-aglycons (dialdehydic forms of elenolic acid linked to tyrosol or hydroxytyrosol) have been identified in the HRGC chromatogram of the phenolic fraction (Angerosa et al., 1995). However, this study has not yet been completed. Total polyphenols and *o*-diphenols were determined colorimetrically using the Folin−Ciocalteu reagent and the Arnow one, respectively. The absorbance data were obtained at 725 and 450 nm wavelength.

Fatty acids, after transformation to methyl esters, were analyzed by HRGC (Ranalli and Serraiocco, 1996a) using a capillary column (25 m, 0.35 mm i.d., 0.25 μm film thickness, from Nordion Ltd., Helsinki, Finland). These components were represented by miristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$), heptadecanoic acid ($C_{17:0}$), heptadecenoic acid ($C_{17:1}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), linolenic acid ($C_{18:3}$), arachidonic acid ($C_{20:0}$), eicosenoic acid ($C_{20:1}$), behenic acid ($C_{22:0}$), and lignoceric acid ($C_{24:0}$).

Sterols and triterpene dialcohols were analyzed by HRGC (Ranalli and Serraiocco, 1996b) using a capillary glass column (25 m, 0.30 mm i.d., 0.20 μm film thickness, from Supelco, Inc., Bellefonte PA). The oil sample was first saponified with an ethanolic KOH solution, the unsaponifiable matter extracted with diethyl ether, and the components separated on a basic silica gel TLC (thin-layer chromatographic) plate using a benzene/acetone (95:5, v/v) mixture as the eluent. The recovered sterols and dialcohols were transformed into trimethylsilyl (TMS) ethers for analysis. Under UV light a mildly basic alcoholic 2,7-dichlorofluoroscein solution was used to reveal the component bands for analysis. A pyridine/hexamethyldisilazane/trimethyl-chlorosilane (9:3:1, v/v/v) mixture was used as a silanizing reagent. Internal standard was α-cholestanol (2 g kg^{-1}, in $CHCl_3$). This fraction included cholesterol (traces), brassicasterol, 24-methylenecholesterol, campesterol, campestanol, stigmasterol, Δ^7-campesterol, Δ^5-23-stigmastadienol, chlerosterol, β-sitosterol, sitostanol, Δ^5-avenasterol, Δ^5-24-stigmastadienol, Δ^7-stigmastenol, and Δ^7-avenasterol. Triterpene dialcohols were represented by erythrodiol and uvaol.

Aliphatic and triterpene alcohols were analyzed by HRGC (Ranalli and Serraiocco, 1996b). The analytical procedure was similar to that for sterols except that alcohol bands instead of sterol bands were recovered by the TLC step. The internal standard was arachidilic alcohol. From the data obtained, the alcoholic index was evaluated (Ranalli and De Mattia, 1997). The aliphatic alcohols identified were 1-docosanol (C_{22}), 1-tetracosanol (C_{24}), 1-hexacosanol (C_{26}), and 1-octacosanol (C_{28}). The triterpene alcohols identified included β-amyrin, butyrospermol, cycloartenol, and 24-methylenecycloartanol.

Triglycerides and diglycerides were analyzed by ^{13}C NMR (nuclear magnetic resonance) (Howarth and Vlahov, 1996; Vlahov, 1996). The oil sample spectra were run in $CDCl_3$ (deuteriochloroform) (250 mg of olive oil/0.5 mL of $CDCl_3$). Free induction decays (FIDs) were acquired at 25 °C using a spectral width of 13 000 Hz. The 131 K acquisition points were zero-filled to 256 K points. A 45° excitation pulse and a 20 s relaxation delay were employed to collect 256 scans. FIDs were processed before Fourier transformation by a Gaussian filter of 0.1 Hz Lorentzian narrowing and 0.15 Hz Gaussian broadening. Chemical shifts were relative to the signal of Me_4Si (tetramethylsilane).

Waxes were analyzed by HRGC (Ranalli et al., 1997) using a SPBTH-5 capillary column (30 m, 0.32 mm i.d., 0.25 μm film thickness, from Supelco Inc., Bellefonte, PA) after their separation with a 70−230 mesh hydrated silica gel column and *n*-hexane/diethyl ether (99:1, v/v) as eluent. The first eluted fraction (~140 mL), with a polarity lower than the triglycerides, was evaporated to dryness and then recovered with *n*-heptane and finally analyzed. The internal standard was lauryl arachidate. This fraction included waxes (C_{34}, C_{36}, C_{38}, C_{40}, C_{42}, C_{44}, and C_{46}).

Stigmastadienes were analyzed by HRGC (Ranalli and Serraiocco, 1996b) using a NB-54 capillary column (25 m, 0.32 mm i.d., 0.10 μm film thickness, from Nordion Ltd., Helsinki, Finland) after their separation with a chromatographic column filled with activated silica 60 and tamponed at the bottom with glass wool and subsequent recovery with hexane eluent. The internal standard was cholestadiene.

Oxidative stability (induction time of the peroxidizing reactions) was determined using a "Rancimat" apparatus (Metrohm Co., Basel, Switzerland), which automatically applied the accelerated Swift's test (120 °C; air flow rate of 20 L/h) (Läubly and Bruttel, 1986).

The other analytical measurements performed were spectrophotometric determination of chlorophylls and pheophytins (green pigments) according to Wolff (1968); spectrophotometric determination of chlorophyll and carotenoid color indices according to Papaseit Totosaus (1986); determination of color ratio (A_{446}/A_{668}) directly on oil; determination of brightness (h %), chroma (σ %), and hue (λd) through transmittance measurements and calculation of integral color index (= σ % log 100/h %) according to Ranalli (1992); titrimetric determination of free acidity and peroxide index; determination of UV spectrophotometric indices according to EEC Regulation No. 2568/91; spectrophotometric determination of carbonyl index; and nephelometric determination of turbidity according to Ranalli and Serraiocco (1996a).

The overall quality index (OQI_1) was evaluated by the algorithm developed by the International Olive Oil Council (IOOC, 1990), based on the acid values, peroxide index, and k_{270} (UV specific extinction at 270 nm wavelength), as well as the sensory score; the other overall quality index (OQI_2) involved also the oil polyphenol content (Solinas et al., 1992).

To perform the quantitative descriptive sensory analyses, the IOOC test included in the EEC Regulation No. 2568/91 was applied by using a standard profile sheet. The gustatory−olfactory−tactile perceptions were evaluated for each sensory attribute using an intensity scale from 0 to 5. A fully trained analytical taste panel recognized by IOOC and made up of 12 assessors and a sensory laboratory were used. All panelists had more than 8 years of experience in evaluating any olive oil types (extra-virgin, virgin, lampant, and refined). Oil samples were heated at 30 °C by a thermostat before sensory analyses and were presented fully randomized to the tasters. Dark-blue glasses were used as no color evaluation was to be made. The magnitude of several sensory attributes, such as "olive fruity" (ripe or green), apple, other ripe fruits, bitter, pungent, sweet, other allowable, sour/winey/vinegary/acid, rough, metallic, mustiness/humidity, muddy sediment, fusty, rancid, and other unallowable, was assessed. Next, an overall evaluation of the magnitudes of positive and negative (off-flavors) attributes was made, and by means of a grading structured scale ranging from 1 to 9 the sensory score was obtained. The oil samples (no. 36) were stored frozen until the moment of the sensory and chemical analyses.

The gas chromatographic analyses were carried out using Mega Series Model 5160 apparatus (Farmitalia C. Erba, Milan, Italy). For the HPLC analyses, a Model Series 3 apparatus (Perkin-Elmer, Norwalk, CT) was used. The spectroscopic observations were obtained by an UV−vis (visible) Perkin-Elmer Model Lambda 2 spectrophotometer. The ^{13}C NMR spectra were obtained using a Bruker Model AC 300 spectrometer (Bruker Instruments, Inc., Karlsruhe, Germany). The turbidity analyses were run on a model ratio turbidimeter (HACH Company, Loveland, CO).

Chemicals were mostly of chromatographic grade and were almost all supplied by Farmitalia C. Erba (Milan, Italy). Bistrimethylsilyltrifluoroacetamide and resorcinol (for HRGC analysis of phenols), Folin−Ciocalteu reagent (for colorimetric analysis of phenols), and silica gel 60 (for HRGC analysis of waxes) were provided by Merck (Darmstadt, Germany). Hexane and chloroform (for HRGC analysis of sterols and waxes) were obtained from Baker (Deventer, Holland), pyridine (for HRGC analysis of sterols) was from Fluka (Buchs, Switzerland), and finally, nonan-1-ol (for HRGC analysis of volatiles) and deuterated chloroform and tetramethylsilane (for ^{13}C NMR analysis of triglycerides and diglycerides) were purchased from Sigma-Aldrich Chemical (St. Louis, MO).

Statistic Analyses. A 2 × 3 factorial design (two extractor kinds × 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, Fischer's LSD test was applied to separate the means (Daniel, 1978). Probabilities higher than $p = 0.05$ were considered nonsignificant. Data concerning analytical oil variables were also processed by principal components analysis (PCA) (Piggot and Jardine, 1986). Barlett's sphericity and Kevin−Meyer−Olkin tests were used to check that PCA could be applied to the data set. PCA was applied under the following conditions: Kaiser's normalization, varimax rotation, and tolerance limits for matrix inversion (0.0001). The cross-validation procedure was used to determine the maximum number of significant dimensions to avoid data over-fitting. The package Unscrambler II Version 5.52 (CAMO A/S, Trondheim, Norway) was used. For Hierarchical Cluster Analysis (HCA), the software SPSS Advanced Statistics 6.1 (SPSS Inc., Chicago, IL) was utilized.

RESULTS AND DISCUSSION

The mean data and standard deviations for the major analytical parameters and indices determined on the oils are presented in Tables 2 and 3.

Headspace Aromatic Components. The content of these substances (which together with many phenolic compounds are mostly responsible for the fruity taste and flavor) was higher in the first extraction oil (Table 2). A similar phenomenon was frequently observed for *trans*-2-hexenal (Table 2), which in good oils is the major component of the volatile fraction (Ranalli and De Mattia, 1997), and for the characteristic ratios of *trans*-2-hexenal/total volatiles and *trans*-2-hexenal/hexanal (data not shown).

According to some authors (Solinas et al., 1987), *trans*-2-hexenal (which forms by enzymic degradation from 13-L-hydroperoxide of linolenic acid) gives a very pleasant odor of fresh cut grass and is responsible for the sensory green-fruity notes of olive oil, together with *cis*-3-hexenyl acetate. According to other authors (Morales et al., 1995), *trans*-2-hexenal is responsible for the bitter almond flavor. In contrast, hexanal gives a very disagreeable smell and is present in small amounts (as are other undesirable volatiles) in good virgin olive oils (Solinas et al., 1988).

Table 2. Analytical and Compositional Characteristics of Virgin Olive Oils of First Extraction (FE) and Second Extraction (SE)[a]

	analytical oil parameters	Leccino		Coratina		Dritta	
		FE	SE	FE	SE	FE	SE
1	total volatiles (as nonan-1-ol, mg kg^{-1})	495.2 (45.8)**	436.6 (32.1)	547.2 (32.1)*	509.1 (38.1)	595.0 (21.2)**	535.5 (34.1)
2	*trans*-2-hexenal (as nonan-1-ol, mg kg^{-1})	276.3 (21.2)**	225.4 (17.0)	350.4 (21.8)*	322.3 (26.5)	297.9 (12.0)	316.9 (16.2)
3	saturated fatty acids (%)[b]	14.9 (0.9)	14.5 (0.9)	11.7 (0.7)	11.2 (0.6)	14.5 (0.4)	14.1 (0.7)
4	unsaturated fatty acids (%)[b]	85.1 (4.8)	85.5 (5.2)	88.3 (3.5)	88.8 (4.1)	85.5 (2.3)	85.9 (3.1)
5	triglycerides (g kg^{-1})[c]	984 (47)	985 (50.0)	985 (41)	988 (52)	982 (47)	982 (55)
6	1,2-diglycerides/1,3-diglycerides ratio	1.0 (0.1)	1.2 (0.1)	1.8 (0.1)	2.0 (0.2)	1.4 (0.1)	1.4 (0.1)
7	waxes ($C_{40} + C_{42} + C_{44} + C_{46}$, mg kg^{-1})	112 (4)*	95 (5)	46 (3)*	31 (2)	77 (4.1)**	57 (4)
8	alcoholic index	0.12 (0.00)*	0.30 (0.00)	0.03 (0.00)*	0.09 (0.01)	0.09 (0.00)	0.14 (0.01)
9	aliphatic alcohols (mg kg^{-1})	93 (4)**	131 (6)	64 (3)*	76 (4)	66 (4)*	82 (5)
10	triterpene alcohols (mg Kg^{-1})	677 (25)**	937 (48)	530 (20)*	584 (36)	759 (37)*	825 (50)
11	total sterols (mg kg^{-1})	1570 (63)	1583 (85)	1195 (57)	1212 (75)	1051 (36)	1091 (45)*
12	campesterol/stigmasterol ratio	2.9 (0.1)*	2.2 (0.2)	5.7 (0.2)	5.5 (0.5)	2.8 (0.1)*	2.5 (0.2)
13	triterpene dialcohols (%)[b]	2.5 (0.1)*	3.0 (0.2)	2.8 (0.1)*	3.1 (0.3)	2.1 (0.1)*	2.4 (0.2)

[a] Data are means of six replicates. Standard deviations are shown in parentheses. Values with one or two asterisks are significantly different from corresponding controls (Fisher's LSD test; *, $p \leq 0.05$; **, $p \leq 0.01$). [b] Related to the total area of the chromatogram. [c] Related to the total glyceridic classes.

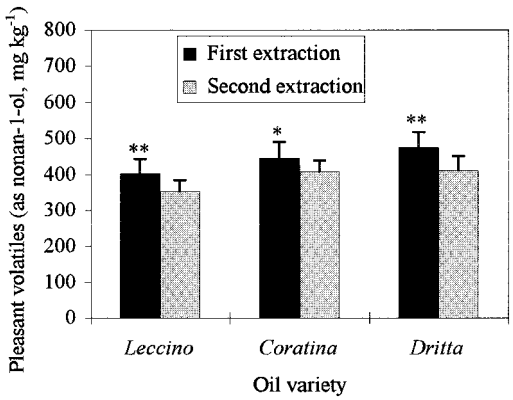

Figure 1. Contents of pleasant volatiles of the oils (means of six replicates). Each value with one or two asterisks is significantly different from the corresponding control (*, $p \leq 0.05$; **, $p \leq 0.01$). Error bars indicate standard deviation.

The content of the other pleasant volatiles (1-penten-3-one, 1-penten-3-ol, 2-penten-1-ol, 1-hexanol, *cis*-3-hexen-1-ol, and *trans*-2-hexenol) identified in oil aromagrams was higher as well in the first extraction product (Figure 1). The data suggest that the diluting lukewarm water used for centrifuging the paste carried away the water-soluble volatiles (alcohols and others). The aromatic composition, however, was primarily affected by genetic factors (olive variety processed) (Table 2).

Phenols and Tocopherols. The content of total phenols and *o*-diphenols was steadily lower in the second extraction oil (Table 3). This was because when water is added to the pastes, which are rich in polyphenols (which are soluble in both water and oil), a significant amount of such constituents is carried away from the oily phase because of partitioning between two nonmixable liquids (Ranalli et al., 1997). A similar trend was observed for hydroxytyrosol (a major free phenol) and for tyrosol-aglycons (hydrolyzable phenols) (Table 3). There was a high linear relationship between values of *o*-diphenols and total phenols (Figure 2).

Phenols and noticeably *o*-diphenols are also natural antioxidants and are largely responsible for the shelf life of virgin olive oil (Ranalli et al., 1997). Only some phenol components (e.g. tyrosol and oleuropein) have no (or negligible) antioxidizing activity (Ranalli and De Mattia, 1997). Recently, we have shown (Ranalli et al., 1997), using a HRGC determination method, that some complex phenols are major components of the phenol fraction of virgin olive oil. Similar results had been obtained by Montedoro et al. (1993) using a spectroscopic determination method.

Tocopherols (which were essentially represented by α-tocopherol and by minute amounts of *β*-, *γ*-, and *δ*-tocopherol), similarly, were present in greater amounts in the first extraction oil (Table 3). These components exert both antioxidant and vitamin action (Ranalli and Angerosa, 1996). The scavenger effect of α-tocopherol on the peroxyl radicals (Schuler, 1990) acts in synergy with the antioxidant activity of hydroxytyrosol and related oleosidic forms (Baldioli et al., 1996).

Sensory Score, Autoxidation Rate, Overall Quality Indices. The autoxidation rate ("Rancimat" test) was regularly lower (in agreement with the higher phenol and tocopherol content) in the first extraction oil (Table 3). There was a good linear relationship between *o*-diphenol content and oxidative stability (Figure 3).

Because of higher phenol and volatile contents, the percolated oil also received a higher sensory score (Table 3). The two oil kinds were without defects, and therefore, qualitative scores were attributed to them only on the basis of their positive organoleptic attributes.

The values (not shown) of overall quality indices were slightly higher in the percolated oil as the values of the parameters involved to calculate them were similar in the two oil types (phenol content and sensory score excepted).

Lipochromes, Chromatic Features, Turbidity. The values (not shown) of σ % (chroma) were mostly higher in the centrifuged oil, whereas the *h* % (brightness) values were lower. The λd (hue) values were similar in the two oil kinds, indicating that both were characterized by a yellow color, which clearly prevailed over green. This was confirmed by values of the color ratio (between carotenoid absorbance and chlorophyll absorbance), which were always >2.5. The concentration of chlorophylls (*a* and *b*) and pheophytins (*a* and *b*) and the values of integral color index and of carotenoid and chlorophyll color indices (Table 3) were also frequently higher in the centrifuged oil and agreed with those of the preceding parameters and with the information given by visible spectra between 400 and 700 nm wavelength. The phenomenon was probably due to the addition of diluting water to the paste, which by means of a mechanical action could favor the contact of oily phase with the vegetable hypoderm tissues (where

Table 3. Other Analytical and Compositional Characteristics of Virgin Olive Oils of First Extraction (FE) and Second Extraction (SE)[a]

	analytical oil parameters	Leccino		Coratina		Dritta	
		FE	SE	FE	SE	FE	SE
14	panel test (score)	7.4 (0.2)**	6.5 (0.5)	7.6 (0.3)*	6.9 (0.5)	7.6 (0.1)*	6.8 (0.5)
15	"Rancimat" stability (h)	11.7 (0.7)**	9.2 (0.9)	15.5 (1.4)*	14.5 (0.9)	9.6 (1.0)**	6.9 (0.5)
16	chlorophylls and pheophytins (mg kg^{-1})	12.4 (0.8)*	15.5 (0.9)	24.0 (1.7)**	28.6 (1.7)	6.4 (0.5)	6.9 (0.6)
17	integral color index	16.5 (0.9)	16.8 (1.4)	21.4 (0.9)**	25.0 (1.1)	11.4 (0.7)*	12.2 (0.7)
18	carbonyl index	4.8 (0.2)**	7.3 (0.5)	3.7 (0.2)*	5.1 (0.4)	2.7 (0.1)*	4.7 (0.4)
19	acidity (as oleic acid, g kg^{-1})	3.0 (0.1)	2.8 (0.2).	6.2 (0.3)	5.6 (0.5)	6.2 (0.3)	5.5 (0.5)
20	peroxide index (mequiv O_2 kg^{-1})	6.6 (0.2)	4.1 (0.3)	5.7 (0.4)	5.4 (0.4)	10.7 (0.7)	9.8 (0.4)
21	k_{232}	1.46 (0.09)	1.30 (0.10)	1.47 (0.10)	1.43 (0.12)	1.34 (0.10)	1.27 (0.12)
22	k_{270}	0.09 (0.00)	0.10 (0.01)	0.15 (0.01)	0.14 (0.01)	0.11 (0.01)	0.06 (0.00)
23	turbidity (NTU)[b]	210 (20)	197 (11)	281 (18)*	250 (18.0)	250 (19)*	220 (15)
24	total tocopherols (mg kg^{-1})	146.6 (7.5)**	122.1 (10.6)	136.8 (9.6)*	119.8 (7.1)	75.4 (3.5)*	64.1 (4.4)
25	total phenols (as caffeic acid, mg kg^{-1})[c]	99 (4)*	86 (5)	203 (8)**	178 (10)	132 (6)*	114 (9)
26	*o*-diphenols (as caffeic acid, mg kg^{-1})[c]	64 (3)*	49 (4)	140 (4)*	121 (9)	88 (3)**	66 (7)
27	tyrosol (as resorcinol, mg kg^{-1})[d]	1.7 (0.1)	1.5 (0.1)	4.5 (0.2)	4.7 (0.4)	5.4 (0.2)	6.7 (0.6)
28	hydroxytyrosol (as resorcinol, mg kg^{-1})[d]	1.0 (0.0)*	0.3 (0.0)	2.8 (0.2)**	0.9 (0.0)	3.3 (0.1)**	0.9 (0.0)
29	tyrosol-aglycons (as resorcinol, mg kg^{-1})[d]	14.4 (0.8)**	6.4 (0.6)	31.3 (1.7)*	25.6 (2.1)	21.7 (1.9)*	18.3 (1.5)
30	hydroxytyrosol-aglycons (as resorcinol, mg kg^{-1})[d]	0.9 (0.0)	1.5 (0.1)	32.6 (1.6)*	27.7 (2.5)	8.8 (0.8)	9.5 (0.8)

[a] Data are means of six replicates. Standard deviations are shown in parentheses. Values with one or two asterisks are significantly different from corresponding controls (Fisher's LSD test; *, $p \leq 0.05$; **, $p \leq 0.01$). [b] NTU, nephelometric turbidity units. [c] As determined by colorimetric method. [d] As determined by HRGC method.

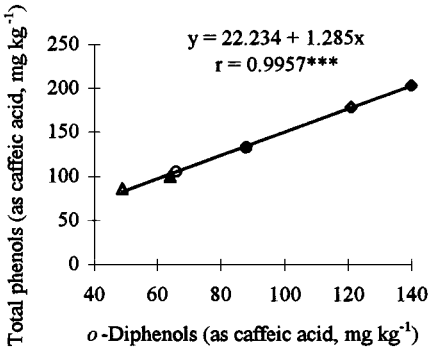

Figure 2. Linear positive relationship between contents of *o*-diphenols and total phenols (both determined colorimetrically) for oils extracted from *Leccino* (▲Δ), *Coratina* (◆◇), and *Dritta* (●○) olive varieties (***, $p \leq 0.0001$). Values are means of six replicates. Standard deviation values are given in Table 3. Solid symbols = first extraction; open symbols = second extraction.

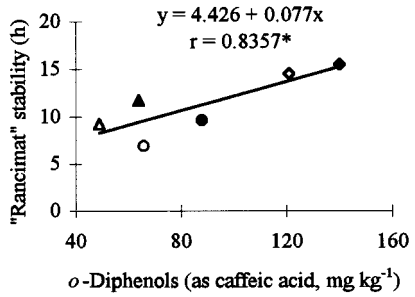

Figure 3. Linear positive relationship between *o*-diphenol content and "Rancimat" stability for oils extracted from *Leccino* (▲Δ), *Coratina* (◆◇), and *Dritta* (●○) olive varieties (*, $p \leq 0.05$). Values are means of six replicates. Standard deviation values are given in Table 3. Solid symbols = first extraction; open symbols = second extraction.

lipochromes are located). In contrast, the values of turbidity were often lower in the second extraction oil (Table 3). Color and turbidity are not considered by the panel test method (EEC Regulation No. 2568/91) for the evaluation of sensory characteristics of virgin olive oil, but they actually influence acceptability of the product by the consumer.

Triacylglycerols, Diacylglycerols, Fatty Acid Composition. Fatty acid composition of the oils was affected by olive variety, but not very much by the extractor kind (Table 2). Total triacylglycerols and total diacylglycerols also were hardly influenced, as well as some glyceridic indices, such as the total diglycerides × 1,3-diglycerides product, the total diglycerides/1,3-diglycerides ratio, and the 1,2-diglycerides/1,3-diglycerides ratio (Table 2). These glyceridic indices are related to quality (Amelotti et al., 1989; Catalano et al., 1994).

However, the determination of some fatty acids (myristic, linolenic, arachidonic, eicosenoic, behenic, and lignoceric acid) and of trilinolein is considered by the EEC Regulation No. 2568/91 to detect the presence of seed oil in virgin olive oil. Also, the determination of total saturated fatty acids in position 2 of triglycerides is considered to detect additions of esterified olive oil to virgin olive oil, and the determination of total *trans*-oleic, *trans*-linoleic, and *trans*-linolenic acid isomers is considered to detect additions of refined olive oil or desterolized seed oil to virgin olive oil and to ascertain whether nonedible olive oil with low acidity has been transformed to edible oil by bland deacidification treatment. Our data confirmed that the *trans*-isomer C_{18} fatty acids are practically absent in genuine olive oil.

Carbonyl Index, Acidity Degree, Peroxide Index, UV Spectrophotometric Indices. Free acidity, peroxide index, UV spectrophotometric indices, and sensory score are the only parameters at present considered by the EEC Regulation No. 2568/91 to evaluate the quality of virgin olive oil.

The specific extinction at 232 nm wavelength, k_{232}, is related to the primary oxidation rate of oil, while k_{270} and Δk (triene peak) are related to the secondary oxidation rate (Ranalli and Angerosa, 1996). The UV spectrophotometric indices are also useful to detect fraudulent additions of refined olive oil to virgin olive oil (Tiscornia, 1992).

Such a group of analytical indices was not affected by the extractor kind (Table 3). Generally, technological treatments induce small variations in these parameters (Ranalli and Angerosa, 1996). Only the values of carbonyl index seemed to be higher in the centrifuged oil (Table 3).

Aliphatic and Triterpene Alcohol Fraction. This fraction includes long-chain alcohols, whose composition

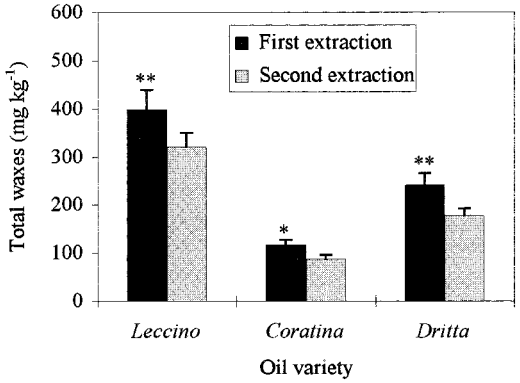

Figure 4. Contents of total waxes of the oils (means of six replicates). Each value with one or two asterisks is significantly different from the corresponding control (*, $p \leq 0.05$; **, $p \leq 0.01$). Error bars indicate standard deviation.

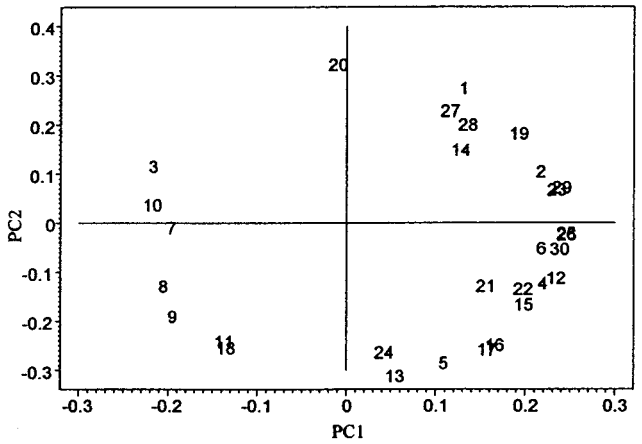

Figure 5. PCA loadings of the main analytical oil variables (Tables 2 and 3) for dimensions 1 and 2.

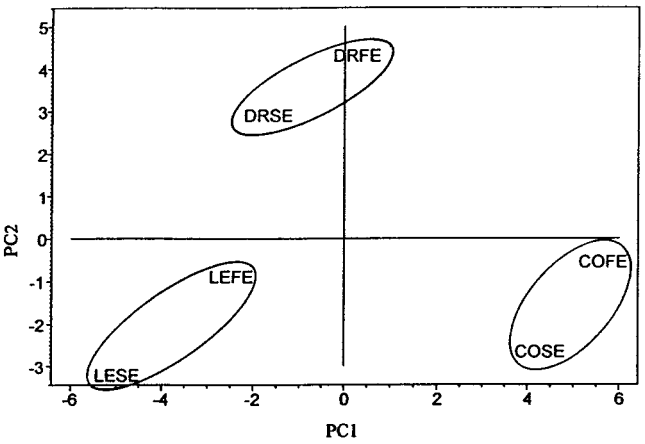

Figure 6. Score plot, by the dimensions 1 and 2 from PCA, of the virgin olive oils of first and second extraction from *Leccino*, *Coratina*, and *Dritta* olive varieties.

is considered useful to assess some genuineness aspect of virgin olive oil (Tiscornia, 1992). The aliphatic and triterpenic alcohol contents were higher in the second extraction oil (Table 2). The values, however, were much lower compared to other second extraction oil kinds produced, carrying out 2 times the milling operation (Ranalli and Serraiocco, 1996a). In this case, in fact, a higher crushing extent of the vegetable tissues is reached, which results in marked solubilization of alcohol constituents in the oil (Ranalli and Serraiocco, 1996b) The values of alcohol index (Camera and Angerosa, 1978), and one inversely related to quality (Ranalli et al., 1997), were frequently hardly higher in the second extraction oil (Table 2).

Steroid Hydrocarbons and Waxes. Analytical data confirmed that no or only traces of steroid hydrocarbons are present in genuine olive oil (Dionisi et al., 1992). These compounds form by degradation of sterols caused by certain technological treatments (refining, desterolization, and others) (Dionisi et al., 1992). In particular, stigmastadienes (isomeric steroid hydrocarbons not resolved by capillary column) are produced by dehydroxylation of the β-sitosterol (major sterol) (Tiscornia, 1992) and are useful in detecting additions of nonvirgin olive oil or desterolized vegetable oil to virgin olive oil (EEC Regulation No. 2568/91).

The first extraction oil was richer in (C_{40} + C_{42} + C_{44} + C_{46}) waxes (Table 2) and total waxes (Figure 4). The phenomenon suggests that these substances, which are present in the external cuticle of olive fruit (Bianchi et al., 1992), were in large part carried away by the percolated oil. The surface lipid layer, which is also present in olive leaves (Bianchi et al., 1993), is believed to be important for chemotaxonomical purposes (Bianchi et al., 1992) and could have a role in determining the resistance of the olive tree to biotic or abiotic stresses (Bianchi et al., 1993).

Additions of husk oil to pressed oil are detected by determining in the mixture the content of waxes or that of triterpene dialcohols or alkanols, as husk oil is rich in these substances (Tiscornia, 1992). Triterpene dialcohols, however, can be removed from the husk oil by strong oxidative treatments, and alkanols can as well, but by other treatments (Dionisi et al., 1992).

Triterpene Dialcohols and Sterols. Relatively higher contents (sometimes not statistically significant) of total sterols and triterpene dialcohols and relatively lower values of the campesterol/stigmasterol ratio (a parameter which is directly related to quality, as stigmasterol content is high in defective oil) (Ranalli and De Mattia, 1997) exhibited the second extraction oil (Table 2). However, the values of triterpene dialcohols were far lower compared to those of second extraction oils produced according to processing cycles including two milling steps (Ranalli and Serraiocco, 1996a). These components are present in elevated concentration in husk oil (which is an inferior oil), therefore they represent a negative index of quality (Ranalli and Serraiocco, 1996b).

Some sterols (cholesterol, brassicasterol, campesterol, stigmasterol, β-sitosterol, and Δ^7-stigmastenol) are considered important for revealing the presence of seed oil or animal olein in virgin olive oil (Dionisi et al., 1992). Determination of total sterols is useful in detecting additions of refined olive oil or desterolized vegetable oil to virgin olive oil (Tiscornia, 1992). The values of sterols, triterpene dialcohols, and other components related to genuineness, in the two oil types were within the limits set by the official normal (EEC Regulation No. 2568/91).

Multivariate Analysis Results. A summary of total variation of the major analytical parameters of oils (Tables 2 and 3) is presented by their factor loadings from the first two factors of the PCA (Figure 5). However, three dimensions of the PCA model were found to be significant and explained 92% fraction of variance.

The first component, accounting for 44% of the total variance, was dominated by the variables total phenols,

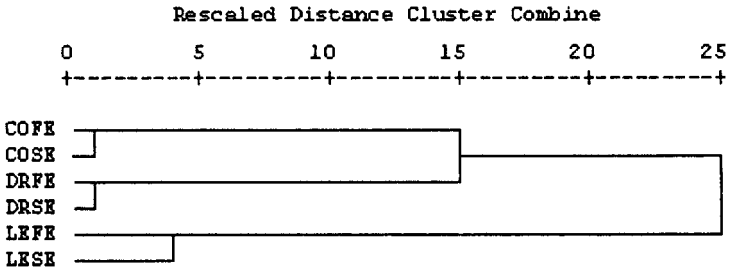

Figure 7. Dendrogram showing the clustering of the *Leccino*, *Coratina*, and *Dritta* olive varieties, independently of extractor kind, using the analytical oil variable data set (Tables 2 and 3).

o-diphenols, tyrosol-aglycons, hydroxytyrosol-aglycons, campesterol/stigmasterol ratio, turbidity, unsaturated fatty acids, *trans*-2-hexenal, 1,2-diglycerides/1,3-diglycerides ratio, triglycerides, k_{270}, acidity, and k_{232} (positively loaded), and the variables triterpene alcohols, saturated fatty acids, alcoholic index, waxes ($C_{40} + C_{42} + C_{44} + C_{46}$), and aliphatic alcohols (negatively loaded).

The second dimension, accounting for 32% of the total variance, was dominated by the variables peroxide index, total volatiles, tyrosol, hydroxytyrosol, acidity, and sensory score (positively loaded) and the variables triterpene dialcohols, triglycerides, total tocopherols, integral color index, chlorophylls and pheophytins, total sterols, and aliphatic alcohols (negatively loaded).

Finally, a third dimension, accounting for 16% of the total variance, was dominated by the variables total volatiles, *trans*-2-hexenal, waxes ($C_{40} + C_{42} + C_{44} + C_{46}$), total tocopherols, total sterols, hydroxytyrosol, and "Rancimat" stability (positively loaded) and the variables tyrosol, 1,2-diglycerides/1,3-diglycerides ratio, triterpene alcohols, and carbonyl index (negatively loaded).

The PCA scores of samples on the 1 and 2 dimensions are given in Figure 6. The two PCA factors were effective in discriminating between oil varieties. There were the *Leccino* and *Dritta* varieties discriminated along the first component (negative half) and the *Coratina* variety along the second component (positive side). The genetic factor (olive variety) effect, as expected, predominated over the extractor type effect. This was confirmed by hierarchical cluster analysis. In fact, the dendrogram (Figure 7) showed three blocks, each consisting of one variety, with a similarity percentage >70% (*Leccino*) and >90% (*Coratina* and *Dritta*).

The comparison between score plot and loading plot indicated that the variables total phenols, *o*-diphenols, hydroxytyrosol-aglycons, 1,2-diglycerides/1,3-diglycerides ratio, campesterol/stigmasterol ratio, "Rancimat" stability, unsaturated fatty acids, k_{232}, k_{270}, clorophylls and pheophytins, and integral color index were mainly responsible for the discrimination of the *Coratina* variety, while the *Dritta* variety was mainly differentiated by the variable peroxide index, and the *Leccino* variety by the variables waxes ($C_{40} + C_{42} + C_{44} + C_{46}$), alcoholic index, aliphatic alcohols, triterpene alcohols, and carbonyl index.

Oil Yields and Analytical Features of Byproducts. The mean oil yield (% w/w, oil fruit basis) of the first extractor was on average 30.5, 31.5, and 37.2% in order with the three olive varieties (*Leccino*, *Coratina*, and *Dritta*), and the corresponding yield of the second extractor was 56.9, 55.5, and 53.3%. There was a significant influence exerted by the olive variety. The differences between the two extractor types were statistically significant ($p \leq 0.01$). The average value of residual oil found in the husk and the vegetable water, regardless of the olive variety processed, was 7.05 and 16.94% dry matter, respectively. These data suggest that the water used in the paste centrifugation step carried away significant amounts of oily pulp particles. The general mean analytical characteristics of wastewater were total phenols (as caffeic acid), 3.4 g L^{-1}; *o*-diphenols (as caffeic acid), 2.1 g L^{-1}; COD (chemical oxygen demand), 102.0 g L^{-1}; turbidity, 27.5×10^3 NTU (nephelometric turbidity units); and dry residue, 88.8 g L^{-1}. These data suggest that the liquid byproduct (effluent) can be a very strong source of pollution.

CONCLUSION

The better quality of the percolated oil did not emerge if the comparison with the centrifuged oil was carried out by evaluating the only analytical official parameters included in the EEC Regulation No. 2568/91. There was the sensory score that was an exception.

The contents of tocopherols, phenols, and aromatic components, which were significantly higher in the percolated oil, have great positive influence on quality, and therefore we propose these parameters are included in the EEC Regulation No. 2568/91. This will enable to define the real commercial grade of the percolated oil.

ABBREVIATIONS USED

HDL, high-density lipoprotein; LDL, low-density lipoprotein; CV, coefficient of variation; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; HRGC, high-resolution gas chromatography; OQI, overall quality index; UV, ultraviolet; vis, visible; EEC, Economic European Community; σ, chroma; *h*, brightness; λd, hue; *k*, specific extinction; Δk, triene peak; COD, chemical oxygen demand; NTU, nephelometric turbidity units; FE, first extraction; SE, second extraction; IOOC, International Olive Oil Council; BSTFA, bis-trimethylsilyltrifluoroacetamide; TMS, trimethylsilyl; TLC, thin-layer chromatography; FIDs, free induction decays; $CDCl_3$, deuteriochloroform; Me_4-Si, tetramethylsilane; PCA, principal components analysis; HCA, hierarchical cluster analysis.

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