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Volatile compounds of virgin olive oil obtained from Italian cultivars grown in Calabria. Effect of processing methods, cultivar, stone removal, and antracnose attack

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Abstract

Calabria is an important olive-growing area of the Mediterranean producing high quality virgin olive oil from a wide variety of cultivars. The present work analyses the influence of cultivars, processing methods, anthracnose attack and stone removal on volatile compounds. Solid phase microextraction (SPME) was used because is easy to use, cheap and rapid. The volatile compound content of different cultivars is influenced by the enzymes involved in the lipoxygenase pathway. The effect of anthracnose attacks increases the content of aldehydes such as heptaldehyde, octyl aldehyde and nonanal. Oil obtained from destoned olives shows a greater quantity of C5 and C6 volatile compounds compared to oil obtained from unstoned olives. Oil obtained in the laboratory has a higher volatile compound content compared to that obtained commercially. Limited scale oil production have a lower oil yields but enables greater content of compounds in the headspace in the final product and thus may be a valid alternative for small agricultural businesses.

Analysis of the principal components is able to distinguish different types of oil.

Keywords: SPME; Olive oil; Volatiles; Stone removal; Antracnose

1. Introduction

Olive oil is one of the oldest known vegetable oils and is the only one that can be consumed in its crude form (unrefined) (Ranalli, Modesti, Patumi, & Fontanazza, 2000). Recent studies have demonstrated the beneficial effects on health of the "Mediterranean diet" based on vegetables, cereals, fish and olive oil (Panagiotakos et al., 2002, Schroder, 2007).

Calabria is an important area of the Mediterranean basin where the climate and soil encourage olive oil production. Indeed, a wide variety of cultivars is successfully grown which produce a high quality of virgin olive oil. This quality is due to both the chemical composition that comprise volatile compounds which, although present in extremely low concentrations, are responsible for the aroma (Zunin et al., 2004).

The concentration of compounds in the volatile fraction depends on the level and activity of enzymes (Angerosa, Basti, Vito, & Lanza, 1999b) which are genetically determinant (Campeol, Flamini, Chericoni, Catalano, & Cremonini, 2001), climate and soil type as well as the fruit ripening cycle (Ranalli et al., 2000; Angerosa et al., 2004). Removal of the stone in order to obtain oil from just the pulp is another factor that influences the volatile

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compounds. A number of authors have studied the volatile composition of oils obtained from whole olives and from olive pulp only (Angerosa et al., 1999b; Amirante, Clodoveo, Dugo, Leone, & Tamborrino, 2006). Olive oil quality is sometimes damaged by anthracnose attacks: the pathogen infects the fruit causing it to rot with adverse effects on the quality and flavour of the oil.

A number of studies have been carried out on the headspace composition of virgin olive oil obtained from typical and non-typical Calabrian cultivars, but a direct comparison between the two is not possible since the oils are obtained from olives with different origin and different processing methods.

Such a complex interplay of factors requires a systematic approach, fixing same variables, to understand the development of volatile compounds of virgin olive oils produced by different cultivars grown in the same soil and climatic conditions, obtained in the laboratory, in commercial process, with anthracnose attack and after stone removal.

2. Materials and methods

2.1. Olive sampling

Samples of virgin oil were obtained from olives of the Leccino, Pendolino, Ciciarello, Nocellara, Coratina, Carolea and Ottobratica cultivars grown in Italy at San Giorgio Morgeto 500 m above sea level – 38° 23′ 0′ N, 16° 6′ 0″ E.

The Leccino, Pendolino, Ciciarello, Nocellara and Coratina cultivars were harvested by hand in order to obtain a homogeneous sample in November 2005.

2.2. Laboratory processed oil

Oil samples were obtained from fruit picked by hand of the Ottobratica (healthy (H), partially damaged (PD) and completely damaged (CD) by anthracnose attacks), Leccino, Pendolino, Ciciarello, Nocellara, Coratina collecting 20 kg of each cultivar at half-black stage and processing the olives within 24 h of harvest to guarantee high quality samples. A laboratory mill equipped with a metal crusher, mixer and press unit was used for oil extraction. The malaxation time was fixed at 30 min. The oil was centrifuged at 3000 rpm for 10 min without adding lukewarm water.

2.3. Commercially processed oil

Carolea and Ottobratica oil was obtained from 500 Kg batches of olives, at half-black stage, harvested by pneumatic and hydraulic shaker, processed within 24 h of harvesting. The olive paste obtained from the mill was malaxed for 30 min at 32 °C and the oil was then extracted using a three-phase centrifugal system with low water added (*two and a half oil decanter*).

Oil from destoned olives was obtained as above substituting the olive crusher with a destoner (Alfalaval).

2.4. Reagents

Hexenal, 1-penten-3-ol, 4-methyl-2-pentanol, 1-heptaldehyde, (E)-2-Hexanal, Hexyl aceatate, Octanal, (Z)-3-hexenylacetate, (E)-2 penten-1-ol, (Z)- 2-penten-1-ol, (E)-2-hexenylacetate, 1-hexanol, (Z)-3-hexen-1-ol, (E)- 3-hexen-1-ol, nonal, (Z)-2-hexen-1-ol, (E)-2-hexen-1-ol from Sigma Aldrich (St. Louis, MO, USA).

2.5. HS-Solid phase microextraction

As reported earlier (Vichi et al., 2003b), 1.5 g of the sample added to 4-methyl-2-pentanol at a concentration of 1.5 μg/g, obtained in deodorized olive oil, was placed in a 10 mL vial sealed with a silicon TFE stopper. After 2 min sample conditioning, the volatile compounds was carried out by exposing the SPME divinylbenzene/Carboxen/polydimethylsiloxane fibre (50/30 μm, 2 cm long from Supelco Ltd., Bellafonte, PA) for 30 min in the headspace of the sample kept in a water bath heated to 40 °C and stirred magnetically.

The fibre was then desorbed for 3 min at 260 °C in a gas chromatograph injection port.

2.6. GC–FID and GC–MS analysis

GC analysis was performed using a thermoelectron GC 8000 series gas chromatograph equipped with an FID detector.

GC–MS analysis was carried out using a Hewlett Packard (Agilent) 5890 coupled to a Hewlett Packard (Agilent) 5973 quadruple mass selective spectrometer. Both were provided with a split–splitless injection port. Helium was used as the gas carrier, at linear velocities of 23 and 17 cm^3/s for GC–FID and GC–MS, respectively.

Separation of compounds was carried out with a Supelcowax-10 (30 m × 0.25 mm i.d., 0.25 μm film thickness) column for GC–MS and a Supelcowax-10 (30 m × 0.25 mm i.d., 0.5 μm film thickness) column for GC–FID, both purchased from Supelco Ltd.

Column temperature was maintained at 40 °C for 10 min and increased to 200 °C at 3 °C/min. The FID temperature was set at 280 °C and the ion source temperature was set at 280 °C.

Electron impact mass spectra were recorded at 70 eV ionization energy in the 15–250 amu mass range at 2 scan/s.

Quantitative analysis of each sample was carried out in triplicate with GC–FID using the internal standard method. The relative response factor was calculated for standard compounds for which a calibration curve was available (Vichi et al., 2003b).

Volatile fraction compounds were identified by comparing their mass spectra with the reference mass spectra of the Wiley 6 library in addition to comparison with retention indices reported in the literature (Vichi et al., 2003b).

2.7. Statistical analysis

The difference in the volatile compound content of oil was tested using Duncan's post-hoc test ($p < 0.05$) on SPSS 12 software (SPSS inc, Illinois). The two-dimensional principal component analysis score plot of volatile compounds data was obtained with Unscrambler 8 software (CAMO PROCESS AS, Norway).

3. Results and discussion

Chemical characterization obtained by analysis of acidity and peroxides shows that all oils except Ottobratica whole, Ottobratica destoned, Ottobratica PD and Ottobratica CD are low in acidity and peroxide value. EC (1991) (Table 1).

3.1. Volatile composition of Leccino, Pendolino, Ciciarello, Nocellara, Coratina laboratory-obtained oil

All oils obtained in the laboratory contain volatile compounds derived from the biochemical pathway of lipoxygenase which allows the catalytic oxidation of 1–4 pentadienic structures to give C6 derivatives (Williams, Salas, Sanchez, & Harwood, 2000; Ridolfi, Terenziani, Patumi, & Fontanazza, 2002). In particular E-2-hexenal, and hexanal, derived from the heterolytic breakdown of the 13-hydroperoxide of linoleic and linolenic acid, respectively, catalysed by enzymes involved in the reaction of hydroperoxide lyase in accordance with published data (Olias, Perez, Rios, & Sanz, 1993; Blèe, 1998). C5 compounds derived from the breakdown of the alkoxy radical are also present (Angerosa, Camera, d'Alessandro, & Mellerio, 1998).

In oil obtained from the Ciciarello and Pendolino varieties, the high level of 1-hexanol and E-2-hexen-1-ol can be attributed to the increased presence or activity of the Alcohol Dehydrogenase (ADH) enzyme. ADH has previously been reported as the enzyme responsible for the biosynthesis of six-carbon alcohols (1-hexanol, E-2-hexanol and Z-3-hexenol) (Olias et al., 1993). In these varieties there is also the effect of the Alcohol Acetyl Transferase (AAT) enzyme which produces hexylacetate from hexanal and Z-3-hexenyl acetate from the corresponding alcohol Z-3-hexenol.

In oil obtained from the Pendolino, Nocellara and Leccino cultivars, the main effect of volatile compounds may be attributed to the ADH enzyme while the AAT enzyme has very little effect.

Data obtained from olive oil of the Coratina variety was highly interesting as it illustrates that in this variety the ADH enzyme has a low activity result in high concentration of E-2-hexenal. The AAT enzyme appears not to be active in this variety, as previously stated in the literature (Angerosa, Basti, & Vito, 1999a).

It is noticeable how the amount of volatile compounds is influenced by enzyme activity as previously reported in the literature (Angerosa et al., 1999a). This is demonstrated by the close correlation (Pearson Correlation = 0.916 at significant 0.05 level) between the concentrations of the E-2-hexen-1-ol/E-2-hexenal and 1-hexanol/hexanal ratios (Table 3), the former substrates and the latter the products of the ADH enzyme.

Table 1
Free fatty acids and peroxide value of analyzed cultivars

Cultivar	Free fatty acids % (oleic acid)	Peroxide value meq/Kg
Leccino	0.4	6
Pendolino	0.1	5
Ciciarello	0.2	6
Coratina	0.1	3
Roggianella	0.3	4
Nocellara	0.2	3
Cassanese	0.8	3
Cannavà	0.2	3
Carolea	0.2	5
Carolea whole	0.3	7
Carolea destoned	0.5	7
Ottobratica whole	0.9	10
Ottobratica destoned	0.9	9
Ottobratica healthy	0.3	5
Ottobratica half damaged	7.7	16
Ottobratica completely damaged	10.0	23

3.2. Ottobratica damaged by anthracnose attacks

An essential factor in obtaining high quality olive oil is the processing of healthy olives. Oil obtained from olives damaged by anthracnose show a significant increase of aldehydes such as heptanal, octanal and nonanal (Table 2) which may be attributed to the decomposition reactions of hydroperoxides formed by the auto-oxidation of oleic acid (Vichi, Pizzale, Conte, Buxaderas, & Lopez-Tamames, 2003a). This is confirmed by the increase in hexanal and the reduction in E-2-hexenal by increasing fruit damage level (Table 2), due to both auto-oxidation and lipoxygenase cascade by the formation of 13-hydroperoxides (Vichi et al., 2003a).

3.3. Effect of stone removal

Data from Table 4 indicates that oil obtained from destoned olives has an accumulation of C5 and C6 compared with oil extracted from whole fruit. This slightly disagrees with previous research data (Angerosa et al., 1999b) that reported a non-significant variation in C5 compounds.

The high quantity of C6 compounds in oil extracted from pulp tissue only may be explained by a greater (Angerosa et al., 1999b) release of membrane-bound enzymes involved in the LOX pathway owing to a higher degree of cellular damage caused by the grinding of pulp tissue in destoned fruits. However, this hypothesis is in contrast to the research of Angerosa et al. (1999b), Reiners and Grosch (1998) who asserts that LOX is the enzyme present in the endosperm of olive fruits.

Table 2
Volatile composition of the different varieties

	Ciciarello	Pendolino	Nocellara	Coratina	Leccino	Ottobratica H	Ottobratica PD	Ottobratica CD
Hydrocarbons								
n-Hexadecan	0.28	–	–	–	–	–	–	–
Aldehydes								
Hexanal	3.15a	15.42d	5.30a,b	9.01c	6.92b,c	14.34d	63.55e	100.55f
Heptanal	0.08a	0.07a	–	0.41c	–	0.27b	1.45d	2.60e
E-2-Hexenal	8.39b	50.76d	2.74a	144.79g	77.51e	94.74f	19.97c	3.25a
Octanal	0.05a	0.04a	0.02b	0.01b	0.05a	0.15c	0.43d	1.01e
E-2-Heptanal	–	–	–	–	–	1.50a	2.79a	4.59a
E,E-2,4-Hexadienal	0.07a	0.10b	–	0.13c	0.12d	0.24e	0.41f	–
E,E-2,4-Heptadienal	–	0.02a	–	–	–	1.00b	–	–
Nonanal	0.07a	0.03a	–	0.07a	0.06a	1.50b	5.25c	12.90d
Decanal	0.01a,b	0.01a,b	0.04b	–	–	–	0.61c	1.53d
E-2-Octenal	–	–	–	–	0.03a	–	0.69b	1.14c
E-2-Nonenal	–	–	–	–	–	–	1.55a	1.79b
Alcohols								
1-Penten-3-ol	0.03a	0.04b	0.07d	0.08e	0.06c	0.08e	0.17f	0.04b
1-Pentanol	–	–	0.06a	–	–	–	–	–
Isoamyl alcohol	–	0.06a		–	–	–	–	–
E-2-Penten-1-ol	0.03a	–	–	–	0.03a	–	–	–
Z-2-Penten-1-ol	0.52b	0.62c	0.75d	0.45a	0.46a	–	–	–
1-Hexanol	3.81c	16.02d	7.37e	–	0.99b	0.61a	0.35a	–
E-3-Hexen-1-ol	0.07a	0.21b	0.42c	0.60d	0.02e	–	–	–
Z-3-Hexen-1-ol	0.67b	1.24d	10.94c		0.44a	0.95	0.55a,b	–
E-2-Hexen-1-ol	7.87e	23.17f	4.27d	0.31a	1.65b	1.95b,c	2.33c	–
Heptanol	–	0.01a	–	–	–	–	–	–
1-Octanol	0.04	–	–	–	–	–	–	–
1-Octen-3-ol	–	–	–	–	–	–	–	0.73
Ketones								
3-Octanone	–	–	–	–	–	–	–	0.39a
2-Octanone	0.04c	0.02 a	0.03b	–	–	–	–	0.55d
Ciclo Hexanone	0.02a	0.03b	–	–	0.01c	–	–	–
2-Nonanone	–	–	–	–	–	–	–	0.23a
6-Methyl-5-Hepten-2-one	0.02a,b	0.02a,b	0.03a,b	–	0.01a,b	0.24b	4.27c	10.78d
E-3-Octen-2-one	–	–	–	–	–	–	–	0.96a
Esters								
Hexyl acetate	0.17a	0.06a	0.02a	–	0.01a	0.36a	–	–
3-Hexen-1-ol acetate	–	–	0.07a	0.07a	–	–	–	–
Z-3-Hexenyl acetate	0.75a	–	–	–	0.10a	0.49a	0.13a	–
Others								
2-Pentyl-furan	–	–	–	–	–	–	0.53a	0.88b
δ-3-Carene	0.56a	–	–	–	–	0.20a	1.01a	–
α-Copaene	–	–	0.04a	–	–	–	–	–
2,2-Ethoxy-ethoxy ethanol	0.08	0.08	–	–	–	–	0.42	0.20

Data values expressed in mg/Kg (cases labelled by different letter are significantly different ($\alpha = 0.05$ significance level).

From a comparison of the data it appears how oil obtained by commercial processes from the Ottobratica cultivar is poor in volatile compounds compared to that obtained in the laboratory as well as that obtained from stoned olives. The cause of this may be the warm water added during the oil extraction phase in the industrial process which may encourage the evaporation of or solubilize the volatile compounds.

3.4. PCA analysis

PCA analysis is used to differentiate production of oil by various type of plant due comparison of volatile compound profiles (Mildner-Szkudlarz, Henryk, Zawirska-Wojtasiak, & Wasowicz, 2003).

Table 3
Value for E-2-Hexen-1ol/E-2-Hexenal and 1-Hexanol/Hexanal concentration ratios

E-2-Hexen-1ol/E-2-Hexenal)	1-Hexanol/Hexanal
0.938	1.210
0.456	1.039
1.558	1.391
0.002	0.000
0.021	0.143
0.021	0.043

Table 4
Volatile composition of oil obtained from whole and destoned olives (cases labelled by different letter are significantly different ($\alpha = 0.05$ significance level)

	Carolea whole	Carolea destoned	Ottobratica whole	Ottobratica destoned
2-Pentanone	10.60b	9.63a	10.59b	9.48a
1-Penten-3-ol	0.00	0.00	0.00	0.02a
1-Pentanol	0.05a	0.23b	0.00	0.00
All C5 compound	10.65	9.86	10.59	9.50
Hexanal	14.38a	17.19b	3.91c	3.27d
E-2-Hexenal	0.11a	1.96b	0.18a	2.28c
Hexyl acetate	0.03a	0.17b	0.15c	0.24d
Z-3-Hexenyl acetate	0.19a	0.62b	0.00	0.52c
1-Hexanol	0.11a	3.27b	0.00	3.01c
Z-3-Hexen-1ol	0.27a	2.33b	0.00	1.04c
E-2-Hexen-1ol	0.00	1.46a	0.00	4.52b
All C6 compound	15.09	27.00	4.24	14.87
2-Pentyl-furan	0.00	0.00	0.00	2.28a
d-3-Carene	0.00	0.00	0.27a	0.21b
2-Octanone	0.02b	0.03a	0.08c	0.03a
Octanal	0.02a	0.04b	0.06c	0.06c
E-2-Heptanal	0.00	0.00	0.00	0.09a
6-Methyl-5-Hepten-2-one	0.02a	0.03b	0.00	0.06c
Nonanal	0.07a	0.16b	0.00	0.59c
Heptanol	0.00	0.00	0.00	0.02a
a-Copaene	0.12b	0.09a	0.00	0.00
2,2-Ethoxy-ethoxy ethanol	0.00	0.03a	0.00	0.36b

The score plot of principal component analysis (PCA) applied to all varieties (Fig. 1) shows a clear discrimination from oil obtained from destoned olives.

PCA analysis is also able to differentiate between oil obtained from a commercial process and oil obtained from olives that have suffered varying degrees of anthracnose attack. The more intense the anthracnose attack on the olives, the more the sample moves to the right of the score plot probably due to high oxidation state of oils and so high level of aldehydes.

The composition of oil headspace is strictly related to the method of production. The laboratory mill processed oil is found in the upper left part of the score plot, while commercially produced oil is found in the lower left part. This is also demonstrated by the difference between the headspace compounds of commercially produced Ottobratica and that obtained in the laboratory.

4. Conclusions

The major components are those C6 compounds derived from LnA compared to those derived from LA. Lipoxygenase enzyme products are those that are generally most present in the volatile fraction of oils obtained both commercially and in the laboratory (Zunin et al., 2004).

Calabrian cultivars Ciciarello and Pendolino have high level of 1-hexanol and E-2-hexen-1-ol; Pendolino, Nocellara and Leccino cultivars show volatile compounds characteristic of the ADH enzyme. Coratina show a high content of E-2-hexenal, that confirm the inactivity of ADH enzyme.

The amount of volatile compounds is influenced by enzyme activity, it can be demonstrated due to high correlation value between the concentrations of the substrates and the products of the enzyme.

Oil obtained from destoned olives by two morphologic different varieties demonstrated that the greater content of volatile compounds compared to that obtained from whole olives is varietal independent.

This methodology could solve the problem of the lack of components in the headspace of some cultivars and there-

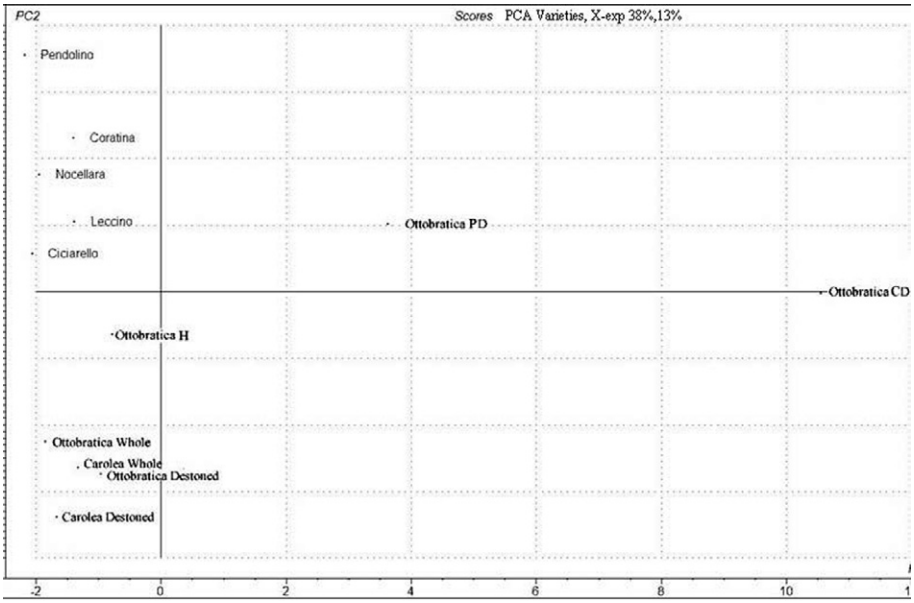

Fig. 1. Score plot of principal component analysis (PCA) applied to all varieties.

fore of flavour for oils obtained from industrial processes in which warm water is added in the extraction phase.

Oil obtained from antracnose attacked olives result in high value of aldehydes hexanal, octanal and nonanal, due to the high oxidation state of oil.

From the results obtained it is not possible to distinguish the oils obtained from different varieties only on the basis of volatile compound content in the headspace. In order to carry out a significant comparison of the varieties the oils must be obtained using the same method.

SPME, GC and PCA methods can differentiate not only plant oils based on the comparison of volatile compound but also discriminate oils obtained from pulp only, antracnose injured fruits and commercial batches.

Limited scale oil production have a lower oil yields but enables greater content of compounds in the headspace in the final product and thus may be a valid alternative for small agricultural businesses.

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