

Virgin olive oil odour notes: their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds

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Received 5 May 1999; received in revised form 2 July 1999; accepted 2 July 1999

Abstract

Fifty-one virgin olive oils of good quality, extracted with a laboratory mill in the same operative conditions from different unripe Italian, Greek and Spanish cultivars, were submitted to sensory evaluation, and to the determination of both polyphenols and the composition of volatile compounds produced through lipoxygenase (LOX) pathways. Univariate analysis was applied to the mentioned variables and some correlations were observed among them. Moreover, a Linear Regression Analysis (LRA) was carried out assuming, as dependent variable, each sensory attribute perceived by tasters and, as independent variables, all compounds derived, derived LOX pathways and polyphenols. The results obtained demonstrate the essential role played by hexanal in the formation of most attributes considered. The most relevant contributors among C₆ compounds from linolenic acid were *trans*-2-hexenal, *trans*-2-hexen-1-ol and *cis*-3-hexenyl acetate. Polyphenols contributed to the characterization of walnut husk, bitter and pungent characters, whereas C₅ compounds, especially 1-penten-3-one, strongly affected most attributes. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Sensory attributes; Volatile compounds; Polyphenols

1. Introduction

Virgin olive oils, extracted from fresh and healthy olive fruits (*Olea europaea* L.) and properly processed, are characterized by a delicate and unique flavour highly appreciated by consumers. Their peculiar delicious taste and aroma are closely related, both to some nonvolatile compounds (Angerosa & Di Giovacchino, 1996; Montedoro, Baldioli & Servili, 1992), and to a number of volatile compounds (Angerosa, Di Giacinto, Vito & Cumitini, 1996; Aparicio, Morales & Alonso, 1996; Morales, Alonso, Rios & Aparicio, 1995). The chemical structures of most nonvolatile and volatile compounds have been assigned by GC–MS (Angerosa, d'Alessandro, Konstantinou & Di Giacinto, 1995; Camera & Solinas, 1990; Morales, Aparicio & Rios, 1994). It has been stated that C₆ compounds, the major components of virgin olive oil headspace, mainly contribute to its green odour notes (Guth & Grosh, 1991; 1993; Morales, Calvente &

Aparicio, 1996). These compounds are produced by a lipoxygenase-mediated oxidation of polyunsaturated fatty acids containing a *cis-cis*-1,4-pentadiene structure during the crushing and malaxation steps of oil production (Hatanaka, Kajiwara & Sekija, 1987; Olias, Pérez, Rios & Sanz, 1993; Vick & Zimmerman, 1987). The malaxation, a low kneading of olive pastes, is an essential technological operation because it helps the small droplets of the oil formed during the milling to merge into large drops that can be easily separated through mechanical systems.

Thus, since sensory quality plays an important role in directing the preference of consumers, many attempts have been made to clarify the relationships between the sensory attributes perceived by assessors in a virgin olive oil and its volatile composition (Aparicio & Morales, 1995; Aparicio et al., 1997; Morales et al., 1995) and polyphenol content (Angerosa & Di Giovacchino, 1996; Gutierrez Rosales, Perdiguero, Gutierrez & Olias, 1992; Montedoro et al., 1992).

Very recently, seven new hydrocarbons were fully characterized as compounds deriving from all the possible

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coupling combinations of the pentene allylic radical (Angerosa, Camera, d'Alessandro & Mellerio, 1998). In addition, the head-spaces of virgin olive oils of high quality showed a reasonable amount of C₅ alcohols and C₅ carbonyl compounds. The simultaneous detection of pentene dimers and C₅ compounds, suggested that the additional branch of the LOX pathway, leading to the production of C₅ compounds through the alkoxy radical, already proved in soy seeds (Salch, Grove, Takamura & Gardner, 1995), was active during olive oil aroma biogenesis (Angerosa et al., 1998).

It was also found that C₅ compounds were contributors to some green sensory perceptions (Morales, Angerosa & Aparicio, in press).

Therefore, the aim of this paper is to explore relationships between green attributes of virgin olive oils and polyphenols and volatile compounds produced by lipoxygenase pathways, including C₅ volatile compounds.

2. Materials and methods

2.1. Materials

All solvents, for organic residual analysis, were purchased from J.T. Baker (Deventer, The Netherlands); activated charcoal (0.5–0.85 mm; 20–35 mesh ASTM) was from E. Merck (Schuchardt, Germany). Charcoal was cleaned by treatment in a Soxhlet apparatus with diethyl ether and tested before the analyses. Hexanal, *cis*-2-penten-1-ol, and *trans*-2-penten-1-ol were purchased from Aldrich (Steinheim, Germany); *cis*-3-hexenyl acetate from Sigma Chemical Co. (St. Louis, USA), *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, *trans*-2-pentenal, 1-penten-3-ol and 1-penten-3-one from Fluka Co. (Buchs, Switzerland), resorcin, bis(trimethylsilyl)trifluoroacetamide (BSTFA), and *trans*-2-hexenal, hexyl acetate, hexan-1-ol from E. Merck (Schuchardt, Germany). Pentene dimers were synthesized from *trans*-3-hexenoic acid according to a previous paper (Angerosa et al., 1998).

2.1.1. Sampling

Fifty-one oil samples were obtained from different cultivars grown in Italy, Greece and Spain during two harvesting years by using a laboratory plant. Olive fruits were crushed with an inox hammer crusher, malaxed for 40 min at 30°C, and the oil was extracted with a basket centrifuge.

2.2. Methods

2.2.1. Sensory analysis

The sensory evaluation of olive oils was performed according to Annex XII of EC regulation no. 2568/91 (EC, 1991). However, the number of descriptors of the official panel was increased to allow a more careful

description of “green” perceptions. With the aim of dissecting and describing different shades of green perceptions, fully trained tasters were requested to assess oil samples extracted from unripe fruits of different cultivars and to describe, freely, the perceptions by means of their own associations with sensations perceived during their own former experiences. After a consensus-building discussion held with assessors to remove possible semantic differences, the descriptors more usually used were introduced in the profile sheet (Fig. 1). Fourteen sensory attributes were considered. The intensity scale ranged from zero to 5. The sample presentation was fully randomized and each sample was evaluated in triplicate.

2.2.2. Dynamic head-space gas chromatography

Volatile compounds, stripped from the oil samples with N₂ (1.2 dm³ min⁻¹, 37°C for 2 h), trapped on 50 mg of activated charcoal and eluted with 1 ml of diethyl ether, were analyzed by GC using a Carlo Erba Mega Series 5160 gas chromatograph equipped with a Nordion silica capillary Carbowax 20 M column (50 m length; 0.32 mm i.d.; 0.5 mm film thickness), an on-column injection system, a CO₂ cryogenic accessory to hold the oven at 25°C and a Flame Ionisation Detector (FID). The oven temperature program was in agreement with the method described in a previous paper (Angerosa, Di Giacinto & d'Alessandro, 1997).

PROFILE SHEET OLFACTORY-GUSTATORY-TACTILE NOTES

DESCRIPTORS	0	1	2	3	4	5
Cut green lawn						
Green leaf or twig						
Green olives						
Wild flowers						
Green banana						
Green tomato						
Almond						
Artichoke						
Apple						
Walnut husk						
Green hay						
Butter/Cream						
Bitter						
Pungent						

- 1 Barely perceptible
- 2 Slight perceptible
- 3 Average
- 4 Great
- 5 Extreme

REMARKS.....

NAME OF ASSESSORS.....

LEGEND OF SAMPLE.....

DATE.....

Fig. 1. Profile sheet: olfactory–gustatory–tactile notes.

2.2.3. GC–MS analysis

The identification of volatile compounds was carried out by GC–MS using the same operative conditions adopted for the gas chromatographic analysis. An HP model 5890A, equipped with an on-column injection system, and coupled with a mass selective detector model HP 5970B, was employed. Volatile compounds were identified by comparison of their mass spectra with those of authentic reference compounds, except for pentene dimers. These last were synthesized from *trans*-3-hexanoic acid and their mass spectra were obtained (Angerosa et al., 1998).

2.2.4. Quantitation

Each C₅ and C₆ compound was quantified on the basis of its gas chromatographic areas using its calibration curve previously drafted. This related known quantities of each C₅ and C₆ compound, added to a fresh refined olive oil, and the corresponding gas chromatographic peak areas (Angerosa et al., 1998; Angerosa, d'Alessandro, Di Girolamo, Vito & Serraiocco, 1997b; Angerosa, Di Giacinto et al., 1997a). The amounts of C₆ and C₅ compounds, expressed as ppm, were the mean values calculated from three independent experiments; the confidence limits were always below 10%.

2.2.5. Evaluation of phenolic compounds

Extraction and purification of phenolic compounds were performed from 30 g of dried virgin olive oil according to a previous paper (Angerosa et al., 1995). The phenolic extract was dissolved in acetone and derivatized with BSTFA, and then submitted to GC–MS analysis (Angerosa et al., 1995). The amounts of secoiridoid compounds were expressed as ppm of resorcin (internal standard).

2.2.6. Statistical analysis

The SPSS (SPSS Inc., Chicago, IL, USA, 1994) software package was applied to datasets to perform descriptive multivariate statistical studies.

3. Results and discussion

Virgin olive oils, extracted with a laboratory mill under the same operative conditions from different unripe Italian, Greek and Spanish cultivars, were submitted either to sensory evaluation, using the profile sheet described in Fig. 1, or to the determination of the composition of volatile compounds and the total amount of secoiridoid compounds by HRGC and GC–MS.

From a sensory point of view all the samples examined belonged to the extra virgin olive oil grade. The direct observation of the intensities of attributes detected by tasters showed that the oils studied were mainly characterized by high intensities of bitter, pungent, fruity (green olives), walnut husk and leaf attributes.

The phenolic content and volatile compound concentrations of the oil samples extracted from different cultivars were different, depending on their genetic constitution. Univariate analysis was applied to sensory attribute intensities, and to concentrations of polyphenols and volatile compounds produced through LOX pathways. Some correlations were observed among the considered variables.

The highest positive correlation (0.91) was found between bitter and pungent attributes. This result was expected since both sensations are generated in the same gustative papillae (Bate-Smith, 1973; Witthead, Bree-man & Kinsella, 1985). In particular, bitter sensation is due to an interaction between polar molecules and the lipid portion of taste papillae membrane (Bate-Smith, 1973) and pungent perception to the stimulation from polar molecules of the trigeminal free endings associated with taste buds in fungiform papillae (Witthead et al., 1985).

It is generally accepted that stimuli mainly responsible for bitter and pungent attributes are tyrosol, hydroxytyrosol and the aglycones that contain them, arising from glycosides naturally occurring in olive fruits (Angerosa et al., 1995; Montedoro et al., 1992). This was confirmed statistically by the positive correlation of polyphenols with bitter (0.60) and pungent (0.57); however, the low levels of correlation underlined that other compounds were also involved in the elicitation of these sensory attributes.

As expected, a negative correlation was found between sweet and bitter (−0.71) and pungent (−0.69) attributes, respectively.

Furthermore, strong positive correlations were detected between leaf and bitter (0.82) and pungent (0.77) attributes, whereas only a lower level of correlation (0.62) was observed between walnut husk and bitter perception.

For a better comprehension of the relationships between polyphenols and volatile compounds arising from the different LOX pathways, and the sensory attributes perceived by tasters, a Linear Regression Analysis (LRA) was applied. LRA was carried out, assuming as dependent variables each sensory attribute perceived by tasters, and, as independent variables, all compounds deriving from LOX pathways and polyphenols, described in Table 1.

A good fit of the model to data was indicated for pungent, bitter and sweet attributes. The low R^2 values detected for apple (0.21), hay (0.35) and artichoke (0.36) suggested that these attributes were mainly elicited by compounds different from those studied. A poorer fit of the model was observed for the remaining odour notes, showing that other additional variables contributed to explain these perceptions. On the other hand, volatiles different from those considered in this work were described by tasters as responsible for these attributes on applying GC-sniffing analysis (Aparicio et al., 1996).

Table 1
R² and regression coefficients of examined variables for each sensory attribute

	Bitter	Pungent	Sweet	Fruity (green olives)	Leaf	Almond	Banana	Lawn	Walnut husk	Flowers	Tomato	Artichoke	Hay	Apple
R ²	0.80	0.80	0.72	0.66	0.65	0.62	0.60	0.57	0.57	0.56	0.51	0.36	0.35	0.21
Hexanal	-0.29	-0.39	0.63	-0.22	-0.46	0.46	0.70	-0.75	-0.08	-0.20	-0.43	-0.05	-0.13	-0.10
Hexan-1-ol	0.04	-0.11	-0.08	0.12	0.16	-0.04	-0.30	0.27	-0.25	0.09	0.64	-0.09	-0.03	0.31
Hexyl acetate	0.08	0.19	0.05	-0.05	-0.10	-0.11	0.09	0.08	0.30	-0.21	-0.09	-0.25	0.17	-0.17
<i>trans</i> -2-Hexenal	-0.17	-0.20	-0.34	0.12	0.03	-0.40	-0.43	0.66	0.06	0.33	-0.02	-0.05	0.39	0.60
<i>trans</i> -2-Hexen-1-ol	-0.17	-0.01	0.32	-0.51	-0.22	-0.01	-0.09	-0.31	0.18	0.68	-0.46	-0.09	-0.18	-0.35
<i>cis</i> -3-Hexen-1-ol	-0.29	-0.15	0.13	0.16	-0.27	-0.27	-0.24	-0.09	-0.21	0.10	0.02	-0.21	0.08	0.07
<i>cis</i> -3-Hexenyl acetate	0.06	-0.06	-0.13	-0.09	-0.17	0.15	0.52	0.25	0.45	0.19	-0.18	0.29	0.08	0.41
<i>trans</i> -2-Pentalen	0.06	0.06	-0.21	-0.38	0.07	0.05	-0.46	-0.21	0.35	0.07	-0.02	0.19	-0.29	-0.07
<i>cis</i> -2-Penten-1-ol	-0.05	0.14	-0.04	0.48	0.03	0.67	-0.41	-0.01	-0.08	0.05	0.11	-0.17	-0.04	-0.12
<i>trans</i> -2-Penten-1-ol	-0.05	-0.09	0.17	-0.07	-0.07	-0.02	0.33	-0.14	0.00	-0.02	-0.03	0.15	-0.12	0.07
1-Penten-3-one	0.53	0.68	-0.06	-0.35	0.45	-0.12	-0.02	0.18	0.04	0.07	0.48	0.27	-0.41	-0.34
1-Penten-3-ol	0.01	-0.14	0.32	0.21	-0.04	-0.30	0.35	-0.16	-0.07	-0.11	-0.41	-0.01	0.59	-0.08
Pentene dimers	0.01	0.02	-0.13	-0.15	-0.26	0.01	-0.02	-0.04	0.26	0.02	-0.26	0.12	0.01	-0.08
Polyphenols	0.40	0.39	-0.12	0.11	0.27	-0.27	-0.13	0.20	0.51	-0.01	-0.29	-0.05	0.23	0.28

Bitter and pungent sensations were positively related to the amount of both 1-penten-3-one and secoiridoid compounds, and negatively to the hexanal content; in addition, *cis*-3-hexen-1-ol accounted for the elicitation of bitter perception. The mentioned correlations could indicate that these attributes were not exclusively due to the stimulation of gustative cells from secoiridoid compounds, as is generally accepted, but that there would be a contribution and/or synergic effect of 1-penten-3-one. This feature was not in agreement with its description (sweet, strawberry) elucidated by assessors by GC sniffing analysis, nor with its characterization by the statistical sensory wheel (Aparicio et al., 1997).

The sweet sensation was mainly dependent on the positive contribution of the hexanal and the negative one of *trans*-2-hexenal and *trans*-2-pentalen; in fact the *trans*-2-hexenal is described by tasters as reminiscent of bitter almond.

Green fruity attribute, the typical basic sensory perception of virgin olive oils, reminiscent of healthy, fresh olive fruits harvested at the right ripening degree, was positively related to *cis*-2-penten-1-ol, whereas negative beta coefficients were detected for *trans*-2-hexen-1-ol and, in a lower proportion, for *trans*-2-pentalen and 1-penten-3-one.

A common negative relationship with hexanal shared the real green perceptions, leaf and lawn. The two odour notes were differentiated by the positive contributors. In particular, positive beta coefficients for 1-penten-3-one and polyphenols were detected for leaf odour note, whereas *trans*-2-hexenal was the most important positive contributor of lawn perception. This result was expected because this compound was reminiscent of freshly cut grass.

Hexanal and *cis*-3-hexenyl acetate were the compounds which more accounted for the elicitation of

banana sensation, negatively affected by *trans*-2-pentalen, *trans*-2-hexenal and *cis*-2-penten-1-ol. These results agreed with the description of this attribute associated by assessors, in the framework of sniffing experiments, with *cis*-3-hexenyl acetate, but not with *cis*-2-penten-1-ol (Aparicio et al., 1997).

Higher absolute values of beta coefficients of C₆ compounds characterized flower attribute, positively related to *trans*-2-hexen-1-ol and negatively with hexyl acetate and hexanal, whereas C₅ compounds — in addition to the C₆ ones — contributed to a tomato odour note. In fact, this last was positively affected by hexan-1-ol and 1-penten-3-one and negatively by *trans*-2-hexen-1-ol, hexanal and 1-penten-3-ol.

The concentration of *cis*-2-pentalen, together with hexanal, positively affected the almond attribute, the sensation that is reminiscent of healthy ripe fruits of almond trees. This attribute correlated negatively with *trans*-2-hexenal and 1-penten-3-ol. It is noteworthy that a negative relationship, even if substantiated by lower beta coefficients, was observed with *cis*-3-hexenol and polyphenols, which are recognized as responsible for green and bitter perceptions, respectively (Bedukian, 1971; Hatanaka, Kajiwara, Horino & Inokuchi, 1992; Montedoro et al., 1992). These results were in agreement with the fact that, sensorially, the absence of grassy and bitter-astringent sensations characterizes the almond attribute. On the other hand, green, bitter almond and astringent sensations well describe walnut husk perception, explained by the high beta values of *cis*-3-hexenyl acetate and *trans*-2-pentalen for green shades (Aparicio et al., 1996) and of secoiridoid compounds for bitter-astringent sensations (Lea & Arnold, 1978; Lee & Lawless, 1991; Naish, Clifford & Birch, 1993). A negative relationship was observed with hexan-1-ol and *cis*-3-hexen-1-ol.

Table 1 underlines that: (i) hexanal played an essential role in the formation of most attributes considered, except for walnut husk; (ii) among C₆ compounds from linolenic acid, *trans*-2-hexenal mainly accounted for lawn, banana and almond, whereas *trans*-2-hexen-1-ol especially accounted for flowers, fruity, and tomato; *cis*-3-hexenyl acetate was the most relevant contributor to banana and walnut husk; (iii) polyphenols contributed to the characterization of walnut husk, bitter and pungent; (iv) C₅ compounds considerably affected most attributes. In particular, 1-penten-3-one was relevant for the elicitation of several attributes, whereas pentene dimers and *trans*-2-penten-1-ol accounted only for leaf, walnut husk, tomato and banana, respectively, with low beta coefficients.

Acknowledgements

This research was supported by the European Union (Project AIR3-CT94 1967), and by the Italian “Ministero delle Politiche Agricole” (MIPA).

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