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Virgin olive oil aroma: Characterization of some Tunisian cultivars

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Abstract

Fruits from four Tunisian cultivars of *Olea europaea* L. grown in the North of Tunisia were handpicked at the same ripening degree and processed with a laboratory mill. The oils were submitted to dynamic headspace and their volatile composition was determined.

The results showed that the most important contributors to olive oil aroma are C_6 aldehydes, alcohols, and esters. These compounds are biogenerated from polyunsaturated fatty acids through the lipoxygenase (LOX) pathway. Furthermore, they pointed out the predominance of the oxidation of linolenic (LnA) acid over linoleic (LA) acid one.

In this predominant part of LOX, the branch A giving rise to *trans*-2-hexenal, *trans*-2-hexenol was more important than the branch B giving rise to *cis*-3-hexenol and *cis*-3-hexenyl acetate. This pointed out that the isomerization of *cis*-3-enals forms to *trans*-2-enal ones is the dominant process of the branch B.

The accumulation of the different metabolites in the oils varied according to cultivar indicating a close dependence on the enzymatic store which is genetically determined.

Keywords: Virgin olive oil; Aroma; Lipoxygenase pathway; C_6 and C_5 compounds; Aldehydes; Alcohols; Esters; Pentene-dimers

1. Introduction

Virgin olive oil is unique because, in contrast to other vegetable oils, it is consumed in its crude state without any refining process (Olias, Pérez, Rios, & Sanz, 1993). Thus, it retains its natural components having a great biological interest such as polyphenols known as powerful antioxidants.

A large increase in demand for virgin olive oil of good quality is due not only to its health virtues but also to its organoleptic properties (Luaces, Pérez, & Sanz, 2003). When properly extracted from fresh and good quality fruits, olive oil provides a delicate and unique aroma highly appreciated by consumers (Angerosa, Di Giacinto, & D'Alessandro, 1997, 2000; Kiritsakis & Christie, 2000). The composition of this aroma resulting of a complex mixture of volatile compounds has been reported (Fedeli, Baroni, & Jacini, 1973; Flath, Forrey, & Guadagni, 1973; Olias, Dobarganes, Guttierez, & Guttierez, 1978).

This complex mixture includes aldehydes, ketones, alcohols and esters (Ridolfi, Terenziani, Patumi, & Fontanazza, 2002). Furthermore, it is well established that aliphatic C_6 compounds (aldehydes, alcohols and their corresponding esters) are the most abundant

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compounds of virgin olive oil aroma (Angerosa, Basti, & Vito, 1999a; Angerosa, Mostallino, Basti, & Vito, 2000). They mainly contribute to its green odour notes (Guth & Grosh, 1993; Morales, Calvente, & Aparicio, 1996).

These compounds are biosynthesized in plants by a lipoxygenase mediated oxidation from C18 unsaturated fatty acids containing *cis–cis* 1,4 pentadiene structure during the crushing and malaxation steps of oil production (Hatanaka, Kajiwara, & Sekiya, 1987; Sanchez & Harwood, 2002; Vick & Zimmermann, 1987).

Many enzymes are involved in this enzymatic pathway (Angerosa, Basti et al., 1999a). They give rise to different amounts of aldehydes, alcohols and esters responsible of the sensory properties and so contributing to the overall virgin olive oil aroma.

According to lipoxygenase pathway, the oxidation of linoleic (LA) and linolenic (Lna) acids gives rise to 9 and 13-hydroperoxide lyase (HPL) of only 13-hydroperoxides (Angerosa, Mostallino, Basti, & Vito, 2001; Olias et al., 1993; Salas & Sanchez, 1998).

In addition to C_6 compounds, the headspace of virgin olive oil shows a reasonable amount of C_5 alcohols and C_5 carbonyl compounds (Angerosa et al., 2000, 2001). The detection of these compounds suggested the presence of an additional branch of the LOX pathway leading to the production of C_5 compounds. This additional branch is active during olive oil aroma biogeneration (Angerosa et al., 2000).

2. Materials and methods

2.1. Sampling

Samples were obtained from homogeneous olive fruits (*Olea europaea* L.) of four Tunisian olive cultivars Chetoui, Chemlali, Meski and Sayali were picked by hand at a known ripening degree. Chetoui and Chemlali cultivars were grown in the locality of Bouargoub in the region of Cap-Bon whereas the cultivars Meski and Sayali were grown in Mornag in the North of Tunisia. Oil samples were obtained by a cold extraction process using a laboratory mill (Institut de l'Olivier) equipped with a metal crusher, a mixer and a basket centrifuge.

2.2. Volatile compound extraction

Fifty grams of oil was put into 120 ml Drechel gas washing bottle with a porous distributor. Volatiles were stripped with nitrogen (1.2 $dm^3\,min^{-1}$, 37 °C) for 2 h, trapped on 50 mg of activated charcoal (0.5–0.85 mm, 20–35 mesh ASTM) from E. Merck (Schuchardt, Germany) and eluted with 1 ml of diethyl ether.

2.3. GC analysis

Gas chromatography was carried out with a Carlo erba mega Series 5160 fitted with a nordion silica capillary carbowx 20 M column (50 m length; 0.32 mm i.d.; 0.5 μm film thickness), and equipped with an on-column injection system, a CO_2 cryogenic accessory to hold the oven at 25 °C and a flame ionization detector (FID). The oven temperature program was run at 25 °C for 7 min, varied at 0.8 °C min^{-1} to 33 °C, then at 2.4 °C min^{-1} to 80 °C and at 3.7 °C min^{-1} to 155 °C holding it there for 20 min. the temperature of the detector was held at 240 °C, with H2 carrier gas at 30 kPa. The injection volume was 0.5 μL. Quantitation was achieved by peak area integration with a Carlo Erba mega series integrator.

2.4. GC-MS analysis

The identification of volatile compounds was done by GC-MS using the same operative conditions adopted for the GC 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 compounds were synthesized from *trans*-2-hexanoic acid and their mass spectra were obtained (Angerosa, D'Alessandro, Basti, & Vito, 1998). Aroma compounds identification was also confirmed by calculation of retention or Kovats indexes.

3. Results and discussion

Aroma is an important criterion for virgin olive oils. Consequently, the identification of the compounds contributing to this aroma is considered as a key for quality and authentication control. In fact, volatile components of olive oil are of a big interest since they are related to its quality and are used to detect adulteration (Cavalli, Fernandez, Lizzani-Cuvelier, & Loiseau, 2004).

Many analytical procedures have been used to isolate, identify and quantify olive oil volatile components. Among techniques with an enrichment step, dynamic headspace, involving the stripping of volatile compounds, their trapping on a suitable adsorbent, their subsequent thermic desorption or elution with a solvent, is the most commonly applied (Angerosa, 2004).

Dynamic headspace is a simple and a fast technique used for food analysis. It is normally used for isolation of products resulting from fatty acid oxidation (Szkudlarz, Jeleń, Zawirska-Wojtasiak, & Wasowicz, 2003).The use of this technique in a great number of studies fostered the analysis and the identification of a

large number of components that contribute to the aroma of olive oil (Kanavouras, Kiritsakis, & Hernandez, 2005; Vichi et al., 2003). Dynamic headspace remains the preferred method for the analysis of virgin olive oil volatiles (Tura, Prenzler, Antolovich, & Robards, 2004).

Angerosa et al. (2004) reported that the most common adsorbents in determining volatile compounds of virgin olive oils are Tenax (Morales, Alonso, Rios, & Aparicio, 1995) and the charcoal (Angerosa et al., 1997; Angerosa, Lanza, Marsilio, & Cumitini, 1999b). Charcoal, in spite of the negative aspect due to a good affinity against water, has been successfully adopted in the determination of volatile fraction of virgin olive oils (Angerosa et al., 2004).

In all oil samples analyzed, the major aroma components were C_6 aldehydes, alcohols and esters. These compounds responsible for green attributes of virgin olive oil are produced through the LOX pathway (Fig. 1) during olive fruit crushing and malaxation and incorporated into resulting oil (Sanchez & Harwood, 2002).

In all the oil samples, the most representative C_6 compounds are aldehydes which accounted for 50.6%, 58.7%, 82.4% and 94.7%, respectively, of the whole C_6 fractions of Chetoui, Meski, Chemlali and Sayali oils (Fig. 2). Furthermore, the percentage of alcohols differed according to the cultivar. It ranged from 4.1% to 42.9%, respectively, in Sayali and Chetoui cultivars. Esters one differed also from a cultivar to another. It

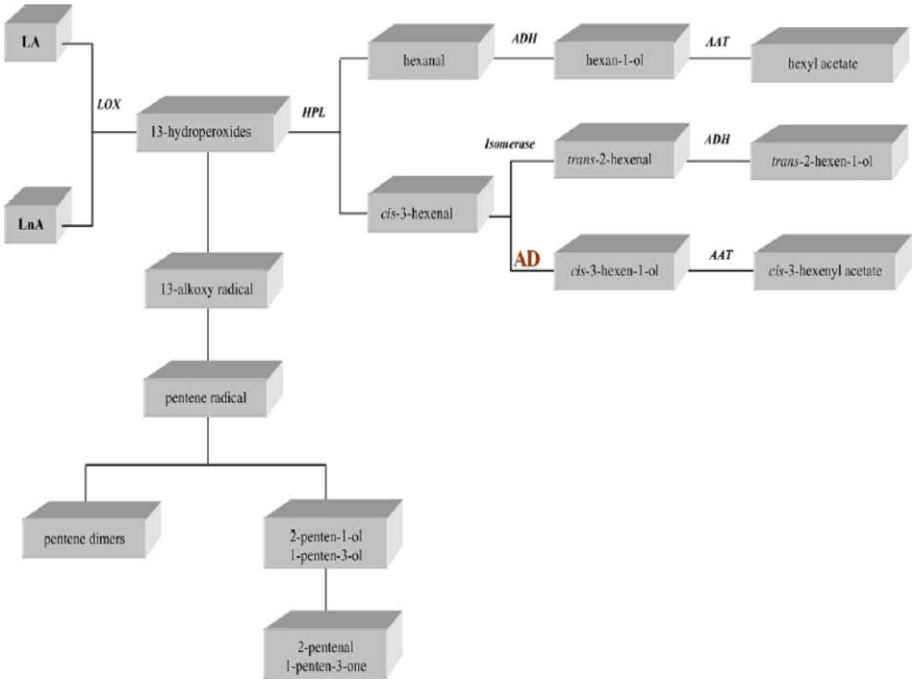

Fig. 1. Lipoxygenase pathway in olive fruit in the production of C_6 and C_5 volatile compounds (Angerosa et al., 2004).

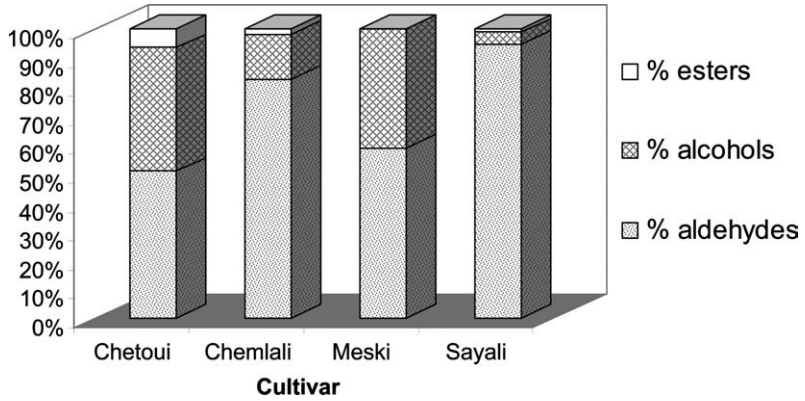

Fig. 2. Distribution of C_6 aldehydes, alcohols and esters in relation to total C_6 compounds in four Tunisian olive cultivars.

Table 1
Amounts of C_6 compounds (ppm of nonan-1-ol) from LOX pathway of four Tunisian olive cultivars

Aroma compounds	R^2	RI	Chetoui	Chemlali	Meski	Sayali
Hexanal	0.16	1099	19.80 ± 4.0	58.32 ± 7.51	20.18 ± 1.67	10.66 ± 5.74
Hexan-1-ol	0.09	1358	19.68 ± 3.51	28.02 ± 2.51	39.32 ± 3.18	4.20 ± 1.50
Hexyl acetate	0.70	1273	6.30 ± 1.82	6.80 ± 1.39	0.37 ± 0.14	1.18 ± 0.14
ΣC_6 from LA	–	–	45.78 ± 9.27	93.14 ± 11.37	59.87 ± 1.66	15.98 ± 7.19
E-2-hexenal	0.35	1213	77.24 ± 6.02	362.20 ± 57.81	152.64 ± 7.87	380.00 ± 30.81
E-2-hexenol	0.06	1411	35.64 ± 5.54	45.16 ± 7.48	78.35 ± 5.35	6.12 ± 0.76
Z-3-hexen-1-ol	0.59	1387	26.8 ± 2.54	6.79 ± 2.65	3.56 ± 1.09	6.60 ± 1.45
Z-3-hexenyl acetate	0.26	1317	6.3 ± 1.93	2.9 ± 0.31	0	3.80 ± 0.43
ΣC_6 from LnA	–	–	145.98 ± 11.05	417.05 ± 68.10	234.55 ± 12.60	396.52 ± 32.12
3-Pentanone	0.11	980	18.02 ± 1.65	17.20 ± 1.80	29.81 ± 2.78	5.21 ± 1.10
1-Penten-3-one	0.58	1018	1.2 ± 0.32	2.60 ± 1.08	1.03 ± 0.21	13.20 ± 2.58
2-Pentenal	0.51	1136	0.89 ± 0.12	3.1 ± 0.95	1.50 ± 0.36	4.20 ± 1.26
1-Penten-3-ol	0.32	1165	3.62 ± 1.28	4.60 ± 1.53	12.29 ± 2.66	6.71 ± 2.13
Pentan-1-ol	0.50	1248	2.12 ± 0.12	1.21 ± 0.33	1.89 ± 0.55	0.72 ± 0.16
Z-2-penten-1-ol	0.32	1320	1.81 ± 0.31	2.84 ± 0.46	0.83 ± 0.22	5.3 ± 1.74
Pentene-dimers	–	–	4.72 ± 1.33	10.85 ± 2.29	16.61 ± 2.82	20.12 ± 3.21

varied from 0.1% to 6.5%, respectively, in Meski and Chetoui cultivars.

Furthermore, in the headspace of all oil samples, the amount of compounds enzymatically produced from the oxidation of linolenic acid (LnA) is greater than the amount of those biogenerated from the oxidation of linoleic acid (LA) (Table 1). Since the pathway with LnA as precursor predomines over the one with LA as precursor in olive oil aroma metabolism, a particular interest was accorded to metabolites deriving from the former.

The compounds generated from the branch A of the linolenic part of the LOX pathway accounted for 77.3%, 97.4%, 97.7% and 98.5% of the totality of C_6 compounds from LnA, respectively, in Chetoui, Sayali, Chemlali and Meski cultivars (Table 2). *Trans*-2-hexenal which the most abundant aroma compound in the four Tunisian virgin olive oils (Table 3) accumulated over *trans*-2-hexenol in all cultivars. This puted in evidence the predominance of the isomerization of *cis*-3-enal forms to *trans*-2-enal ones in the branch A (Angerosa, Basti et al., 1999a). Chetoui and Meski cultivars showed exceptional important activities since *trans*-2-hexenol accounted for 24.4% and 33.6%, respectively, in these Tunisian cultivars.

Cis-3-hexenyl acetate is present in aroma of Chemlali and sayali oils at a low amount, thus indicating a low presence of alcohol acyl transferase (AAT). Higher level of AAT was evidenced in Chetoui cultivar since the quantity of *cis*-3-hexenyl acetate is more important than in Chemlai and Sayali oils. In Meski oil headspace, *cis*-3-hexenyl acetate was completely absent pointing out absence of activity AAT.

In addition of LOX compounds, the aroma of Tunisian virgin olive oils contains reasonable amounts of pentene dimers and C_5 compounds (Table 1). The detection of these compounds indicates the existence of an additional branch of the LOX pathway which leads to the production of C_5 compounds through the alkoxy radical (Fig. 1) already proven in soy seeds (Salch, grove, Takamura, & Gardner, 1995).

Each one of the aroma compounds of virgin olive oil is related to one or various sensory attributes. In fact, Angerosa et al. (2000) reported that E-2-hexenal mainly contributes to lawn, banana and almond notes whereas E-2-hexenol accounts for flowers, fruity and tomato

Table 2
Distribution of the two branches of the LOX pathway part having LnA as precursor in four Tunisian olive cultivars

	Chetoui	Chemlali	Meski	Sayali
% Branch A/ΣC_6 from LnA	77.6	97.7	98.5	97.4
% Branch B/ΣC_6 from LnA	22.4	2.3	1.5	2.6

Table 3
Distribution of each C_6 compound in relation to the whole C_6 compounds

	Chetoui	Chemlali	Meski	Sayali
% Hexanal/ΣC_6compounds	10.3 ± 1.01	11.5 ± 0.33	6.9 ± 0.93	2.5 ± 1.17
% Hexanol/ΣC_6compounds	10.2 ± 0.86	5.5 ± 0.37	13.3 ± 0.39	1.0 ± 0.27
% Hexyl acetate/ΣC_6compounds	3.3 ± 0.61	1.3 ± 0.07	0.1 ± 0.04	0.3 ± 0.04
% E-2-hexenal/ΣC_6compounds	40.3 ± 1.13	71.0 ± 0.28	51.8 ± 0.26	92.2 ± 1.58
% E-2-hexenol/ΣC_6compounds	18.5 ± 0.93	8.8 ± 0.11	26.6 ± 0.40	1.5 ± 0.10
% Z-3-hexenol/ΣC_6compounds	14.1 ± 2.84	1.3 ± 0.32	1.2 ± 0.30	1.6 ± 0.21
% Z-3-hexenyl acetateΣC_6compounds	3.2 ± 0.66	0.6 ± 0.08	0.0	0.9 ± 0.03

ones. Z-3-hexenyl acetate is the most important contributor to banana and walnut husk notes. The same authors reported also that C_5 compounds considerably affect most attributes.

These results pointed out the evidence of a strict dependence of olive oil aroma on the enzymatic store which is genetically determined (Angerosa, Basti et al., 1999a). Furthermore, it is important to mention that Tunisian olive oils had a volatile composition which is similar to European ones. This feature indicates that LOX pathway had the same importance and is the predominant pathway of volatiles biogeneration in Tunisian and European virgin olive oils. It is not the case for Australian virgin olive oils in which LOX pathway had not the same importance and whose major aroma compounds are not always C_6 ones (Tura et al., 2004).

All these results put in evidence that olive oil aroma compounds accumulate differently according to the cultivar. In fact, accumulation of these metabolites has a close dependence on the enzymatic store which is genetically determined, according to results of other researches (Angerosa, Basti et al., 1999a).

In a future work, we will try to complete this characterization by the study of the effects of other parameters such as environmental conditions, extraction procedure and ripening degree on the aroma composition and its sensorial quality.

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