Virgin Olive Oil Volatile Compounds from Lipoxygenase Pathway and Characterization of Some Italian Cultivars

F. Angerosa,* C. Basti, and R. Vito

Istituto Sperimentale per la Elaiotecnica - Contr. Fonte Umano, 65013 Città S. Angelo (PE), Italy

Fruits from seven different varieties of *Olea europaea* L., grown in the same environmental conditions, were harvested in two succeeding years at the same ripening degree and immediately processed. The oils obtained were submitted to gas chromatographic determination of the volatile compounds extracted by dynamic headspace technique. The results demonstrated that the accumulation of the different metabolites in the oils obtained from the various cultivars were strictly connected with the varietal parameter because of the enzyme differences genetically determined. This feature made possible the differentiation of the examined cultivars on the basis of the percent of each metabolite from the enzymatic transformation of 13-hydroperoxides of linolenic acid. Oils from Picual and Koroneiki varieties cultivated in Spain and Greece, respectively, showed contents of volatiles very similar to those detected in the oils of the same varieties cultivated in Italy, proving that they were not significantly influenced by the environmental conditions.

Keywords: *Olive oil; C_6 volatile compounds; cultivar differentiation*

INTRODUCTION

Volatile compounds responsible for green attributes of virgin olive oils have been proved to be produced through the enzymatic oxidation of linolenic and linoleic acids (Vick and Zimmerman, 1987; Hatanaka, 1993; Olias et al., 1993).

The qualitative and quantitative composition of accumulation products is tightly dependent on the levels of enzymes involved in the pathway and on their activity. Enzyme contents are genetically determined, but the production of metabolites changes in relation to the ripening degree and storage time of fruits and the operative conditions used during the oil extraction (Montedoro and Garofolo, 1984; Angerosa et al., 1989; Angerosa and Di Giacinto, 1995; Angerosa et al., 1998).

The most important modifications of the qualitative and quantitative composition of most oil fractions (Mariani et al., 1991; Solinas 1987; Montedoro and Servili 1992; Minguez-Mozquera and Gallardo-Guerrero, 1995) have been observed during the olive ripening process. In the framework of these changes the content of each volatile arising from the lipoxygenase (LOX) pathway also shows a different evolution pattern in relation to the extension of fruit pigmentation (Montedoro and Garofolo, 1984; Solinas et al., 1987; Guth and Grosch, 1993).

Olive fruit preservation, even if carried out in ideal conditions (low temperature and very thin layer of olives), causes a decrease in concentration of compounds from the LOX pathway (Angerosa et al., 1989). The content of all those compounds decreases by 30−40% in oils obtained from olives preserved for 15 days in relation to their level in the oil from fresh fruits (Angerosa et al., 1989). The decrease becomes more evident for longer preservation times.

Technological conditions during the oil extraction influence the volatile composition. Oils extracted from a stone mill have been found to contain higher level of volatiles than samples obtained from a metallic crusher, while the time of olive paste malaxation promotes the accumulation of volatiles (Angerosa and Di Giacinto, 1995; Angerosa et al., 1998).

On the other hand, the fruit growing environment could produce some effects, perhaps indirectly modifying the ripening degree of fruits. In fact the environmental conditions affect some oil fractions, as the results of previous investigations have evidenced (Montedoro et al., 1989; Pannelli et al., 1990; Angerosa et al., 1996).

The aim of this research was to determine changes of the composition of C_6 aldehydes, alcohols, and esters from the LOX pathway due exclusively to the varietal factor.

MATERIALS AND METHODS

Olives from Leccino, Coratina, Provenzale, Carolea, Gentile di Chieti, Picual, and Koroneiki varieties, cultivated in the experimental orchard of the Institute, were picked by hand during two succeeding harvesting years (1996 and 1997). Healthy fruits were arranged so that all samples showed the same ripening degree. The ripening characteristics were the following: 25% green; 25% yellow; 25% partially turning, and 25% completely turned to purple. The olives immediately after their harvesting were deleafed, washed, and crushed using an inox hammer mill. The centrifugation of pastes, malaxed at 30 °C for 40 min, was performed using a basket centrifuge.

Volatile compounds were extracted from oil samples by dynamic headspace technique and analyzed by high-resolution gas chromatography (HRGC) on a silica capillary Carbowax 20M column according to the operative conditions described in a previous paper (Angerosa et al., 1997). C_6 compounds were quantified on the basis of their gas chromatographic areas using calibration curves previously drafted. These last related known quantities of the different C_6 compounds, added to a fresh refined olive oil, and the corresponding gas chromatographic peak areas (Angerosa et al., 1997).

In addition oils obtained from fruits of Picual and Koroneki varieties, cultivated in Spain and Greece, respectively, and picked by hand with a similar ripening degree in 1996 were analyzed.

* Author to whom correspondence should be addressed (telephone +39 85 95212; fax +39 085 959518; e-mail elaiotec@unich.it.

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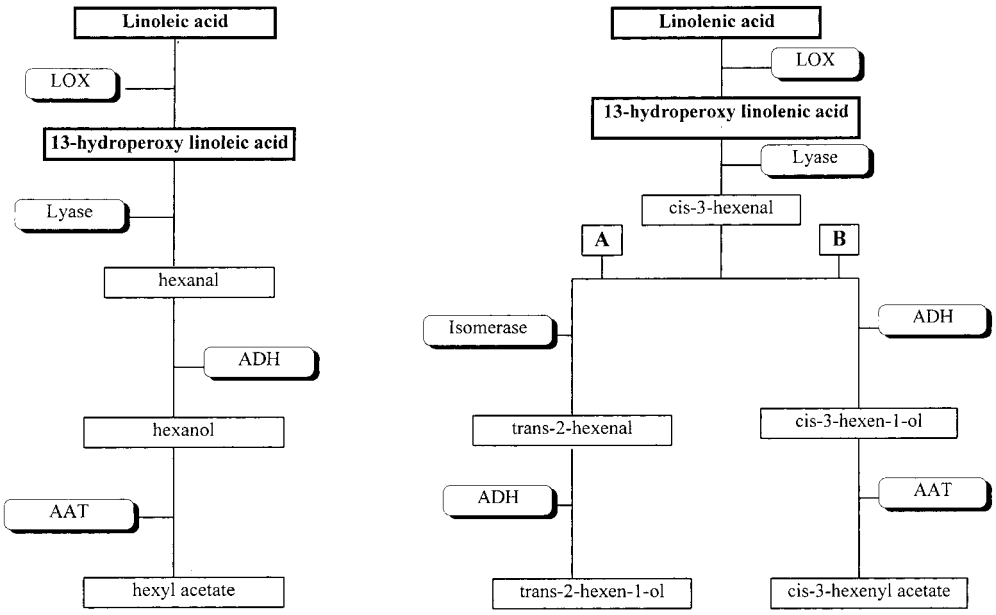

Figure 1. Pathways of the enzymatic oxidation of linoleic and linolenic acids.

Table 1. ppm of C_6 Compounds from LOX Pathways of Seven Different Olive Cultivars Measured during Two Harvesting Years

	ppm													
	Leccino		Coratina		Provenzale		Carolea		Gentile di Chieti		Picual		Koroneiki	
	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
hexanal	2.0	3.5	2.3	2.6	2.1	1.8	0.8	0.6	1.4	1.1	2.0	1.4	0.9	1.1
hexan-1-ol	2.2	0.7	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.4
hexyl acetate	0.0	0.0	0.0	0.0	0.2	0.3	0.0	0.0	0.2	0.1	0.1	0.1	0.4	0.3
total amount C_6 comp. from LA	4.2	4.2	2.5	2.7	2.4	2.1	0.9	0.7	1.7	1.3	2.2	1.6	1.4	1.8
trans-2-hexenal	53.0	41.0	43.4	43.8	5.6	5.6	6.9	7.7	7.0	6.0	25.0	19.6	4.9	4.4
trans-2-hexen-1-ol	6.3	4.9	0.7	0.6	0.1	0.1	0.2	0.2	0.1	0.3	0.3	0.3	0.3	0.2
cis-3-hexen-1-ol	0.5	0.5	0.3	0.3	0.8	0.7	1.0	1.5	1.1	1.6	1.6	1.0	1.0	1.3
cis-3-hexenyl acetate	0.0	0.0	0.0	0.0	0.7	0.7	0.0	0.0	0.8	0.7	0.3	0.3	1.6	1.7
total amount C_6 comp. from LnA	59.8	46.4	44.4	44.7	7.2	7.1	8.1	9.4	9.0	8.6	27.2	21.2	8.8	7.6
% C_6 aldehydes/ΣC_6	85.9	87.9	97.4	97.9	80.2	80.4	85.6	82.2	78.5	71.7	91.8	92.9	63.0	58.5
% C_6 alcohols/ΣC_6	14.1	12.1	2.6	2.1	10.4	8.7	14.4	17.8	12.2	20.2	6.8	15.2	15.2	20.2
% C_6 esters/ΣC_6	0.0	0.0	0.0	0.0	9.4	10.9	0.0	0.0	9.3	8.1	1.4	0.9	21.7	21.3

RESULTS AND DISCUSSION

The effect of the cultivar on the C_6 volatile composition has been studied considering oils from seven varieties, chosen from among the most representative of the Mediterranean basin oil production. Experiments were planned so that all differences in variability of volatile composition not attributable to varietal factor could be removed. With this aim the olives from all varieties studied, cultivated in the same environmental conditions, have been harvested with a fixed ripening degree, based on the visual evaluation of the pigmentation extension, and processed in the same operative conditions. The oils obtained were submitted to gas chromatographic analysis of the dynamic headspace components.

It is known that lipoxygenases, after their release owing to disruption of fruit cells during the milling, produce 9- and 13-hydroperoxides of linolenic and linoleic acids in a ratio ranging between 65:35 and 55:45, respectively (Olias et al., 1993). The lack of C_9 metabolites in the volatile fraction of virgin olive oils implies that only 13-hydroperoxides are the substrate for further enzymatic reactions.

13-Hydroperoxides of linoleic acid are cleaved by hydroperoxide lyases (HPL) producing hexanal, which is reduced to hexanol by alcohol dehydrogenases (ADH). The hexyl acetate is formed (Figure 1) from hexanol because of the catalytic activity of alcohol acetyl transferases (AAT).

The metabolism of 13-hydroperoxides of linolenic acid is more complicated. Their cleavage mediated by HPL gives rise to *cis*-3-hexenal. This metabolite does not accumulate in the volatile fraction of virgin olive oils, since it has been detected only at very low level (Guth and Grosch, 1993). Besides, the incubation of olive fruit crude extract with 13-hydroperoxides of linolenic acid produces only *trans*-2-hexenal (Olias et al., 1993). It is either quickly enzymatically reduced to *cis*-3-hexen-1-ol and subsequently transformed into its corresponding ester (B branch of Figure 1) or is isomerized to *trans*-2-hexenal and in the next step reduced to *trans*-2-hexen-1-ol (A branch of Figure 1).

Hydroperoxide lyases, alcohol dehydrogenases, and alcohol acetyl transferases have been evidenced in milled olives, and the activity of these enzymes, partially purified, has been measured (Olias et al., 1993). On the other hand, the presence of isomerases catalyzing the isomerization of a *cis*-3-enal to the more common *trans*-2-enal has only been demonstrated in cucumber (Phillips et al., 1979) and has only been proposed to occur in other plants (Olias et al., 1993). Therefore, it cannot be rejected a priori that a chemical isomerization can occur.

Table 2. Micromoles of C_6 Metabolites from LOX Pathway, and Their Percent Distribution in Relation to Their Total Amount

		Leccino		Coratina		Provenzale		Carolea		Gentile di Chieti		Picual			Koroneiki		
branch		1996	1997	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997	Spain	1996	1997	Greece
	trans-2-hexenal	540.0	417.8	442.2	446.3	57.1	57.1	70.3	78.5	71.3	61.1	254.7	199.7	238.4	49.9	44.8	36.7
A	*trans*-2-hexen-1-ol	62.9	48.9	7.0	6.0	1.0	1.0	2.0	2.0	1.0	3.0	3.0	3.0	7.0	3.0	2.0	1.0
	cis-3-hexen-1-ol	5.0	5.0	3.0	3.0	8.0	7.0	10.0	15.0	11.0	16.0	16.0	10.0	2.0	10.0	13.0	5.0
B	*cis*-3-hexenyl acetate	0.0	0.0	0.0	0.0	4.9	4.9	0.0	0.0	5.6	4.9	2.1	2.1	0.7	11.3	12.0	12.0
	% branch A/ΣC6 from Lna	99.2	98.9	99.3	99.3	81.7	82.9	87.8	84.4	80.9	75.3	93.8	94.4	96.9	71.6	65.3	68.9
	% branch B/ΣC6 from Lna	0.8	1.1	0.7	0.7	18.3	17.1	12,2	15,6	19.1	24.7	6.2	5.6	3.1	28.4	34.7	31.1
	% 2-hexenal/ΣC6	88.9	88.5	97.8	98.0	80.3	81.5	85.4	82.3	79.8	71,8	92.7	93.0	95.6	67.5	65.2	67.1
A	% 2-hexen-1-ol/ΣC6	10.3	10.4	1.5	1.3	1.4	1.4	2.4	2.5	1.1	3,5	1.1	1,.4	1.2	4.1	2.8	1.8
	% 3-hexen-1-ol/ΣC6	0.8	1.1	0.7	0.7	11.3	10.0	12.2	15.2	12.4	18.8	5,.5	4.7	2.8	13.5	18.1	9.1
B	% 3-hexenyil acetate/ΣC6	0.0	0.0	0.0	0.0	7.0	7.1	tr	tr	6.7	5.9	0.7	0.9	0.4	14.9	16.7	21.9

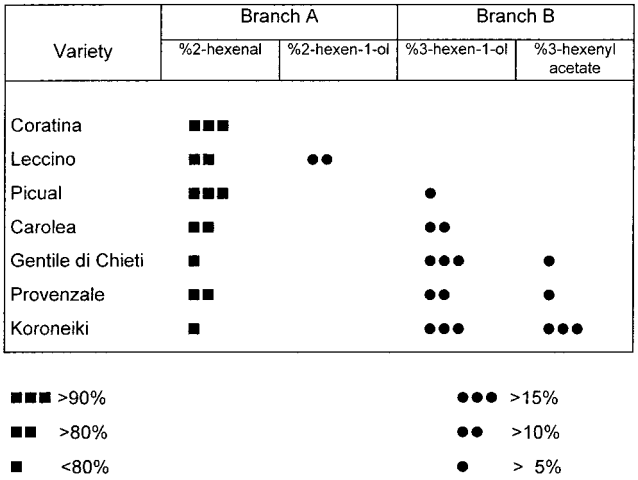

Figure 2. Accumulation of the main metabolites coming from the enzymatic oxidation of linolenic acid, expressed as percent in relation to their total amount, in the seven different cultivars.

The quantitative composition, expressed as ppm, of all C_6 compounds produced by the LOX pathway in oils from each variety for the 2 years is shown in Table 1. The first thing to be noted observing data of this table was the accumulation in all cases of a greater quantity of unsaturated compounds having linolenic acid (LnA) as a precursor over saturated ones deriving from linoleic acid (LA). These results are in agreement with the greater preference shown by alcohol dehydrogenases and alcohol acetyl transferases of olive fruits for unsaturated metabolites (Olias et al., 1993).

The major components of the C_6 fraction are, in all oil samples, aldehydes, which represent from 58.5% (Koroneiki cv) to 97.9% (Coratina cv) of all C_6 compounds. The percentages of alcohols and esters differ respectively for each variety evidencing a strict dependence on the enzymatic store.

The pathway having LnA as a precursor leads to four metabolites through two possible branches (Figure 1) with the mediation of a greater number of enzymes than the LA pathway. This feature together with the really low level of volatiles coming from LA prompted us to consider only changes of C_6 compounds from LnA.

The importance of the A branch over the B branch could be established expressing as micromoles the content of all accumulation products arising from the enzymatic oxidation of LnA and calculating the percent of these metabolites. In fact their total quantity represented the amount of their common precursor *cis*-3-hexenal. In our gas chromatographic conditions the *cis*-3-hexenal could not be completely separated from the *trans*-2-hexenal because their retention times are very similar, and therefore the amount of *trans*-2-hexenal reported in Table 2 includes the low quantity of *cis*-3-hexenal (Guth and Grosch, 1993) that did not undergo isomerization.

The A branch was predominant in all the varieties considered and was the principal pathway active for the Leccino and Coratina cultivars. In contrast, the simultaneous presence of metabolites arising from A and B branches was observed in Carolea, Gentile di Chieti, Provenzale, and Koroneiki varieties. Again, the A branch was predominant, whereas the B branch accounted for 12.2% for Carolea in 1996 to 34.7% for Koroneiki in 1997. In Picual cultivar there was a low accumulation (5.6−6.2%) of metabolites of the B branch.

The accumulation of *trans*-2-hexenal over trans 2-hexen-1-ol in all varieties studied clearly pointed out that the isomerization of *cis*-3-enal forms to *trans*-2-enal ones was always the dominant process of the A branch. Only the Leccino variety showed some ADH activity since percentages of about 10% of *trans*-2-hexen-1-ol in relation to all C_6 compounds from LnA were observed.

The B branch was active in a different way in all varieties, except for Leccino and Coratina, as mentioned above. In all other varieties the enzymatic reduction of *cis*-3-hexenal to *cis*-3-hexen-1-ol was the main step of the B branch.

The trace levels of *cis*-3-hexenyl acetate in Carolea indicated a very low content of alcohol acetyl transferases catalyzing the production of this ester. A negligible accumulation of *cis*-3-hexenyl acetate was evidenced in Picual cultivar pointing out in this variety a low presence of AATs. The greater quantities (from 6 to 16 times) of *cis*-3-hexenyl acetate observed in Gentile di Chieti, Provenzale, and Koroneiki indicated higher levels of AATs in these varieties, in particular in Koroneiki cultivar.

The different accumulation of the main metabolites coming from the enzymatic oxidation of LnA, being tightly connected with the levels of the enzymes genetically determined, allowed one to distinguish the varieties considered, as shown in Figure 2. In fact each cultivar was characterized in the 2 harvesting years by the same distribution of metabolites (Table 2). For example, in the cultivar Coratina *trans*-2-hexenal accounted for over 90% of all C_6 compounds and had insignificant levels of the other compounds. Nevertheless, different contents of each metabolite could be observed in relation to the other parameters mentioned in the Introduction. This feature occurred even though the climatic variables were different in the 2 harvesting years, as evidenced in Figure 3 for temperatures and in Figure 4 for the rainfall. With respect to minimum temperatures, the year 1996 was generally warmer than 1997; the average maximum temperatures were quite

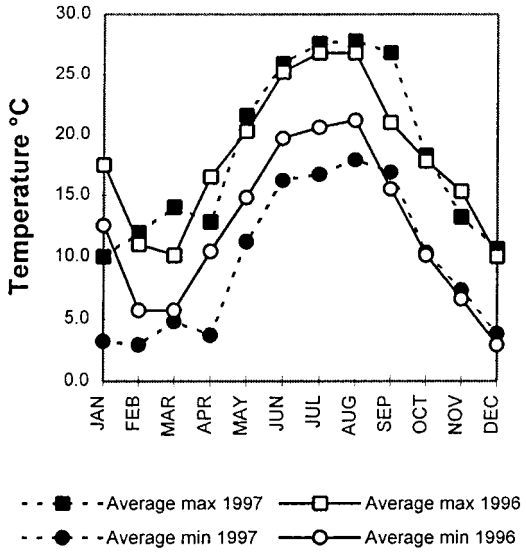

Figure 3. Average maximum and minimum temperature recorded during the years 1996 and 1997 in the growing environment of examined cultivars.

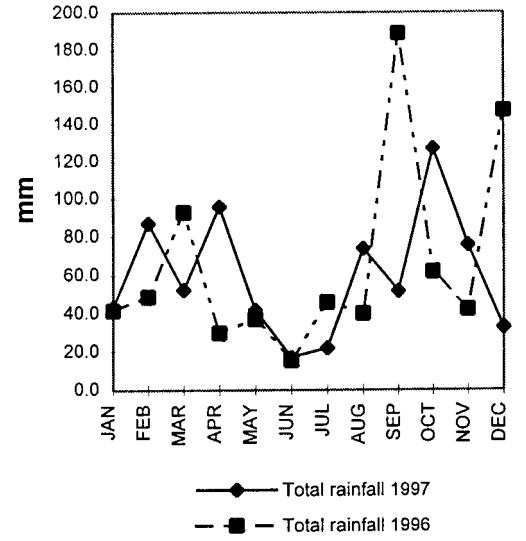

Figure 4. Total rainfall, as mm, recorded during the years 1996 and 1997 in the growing environment of examined cultivars.

similar, except for the month of September 1997 that was exceptionally hot. The total amount of rainfall in the 2 years was similar, but its distribution was different since the rains were mainly concentrated in September and December for 1996.

The similar behavior of oils obtained from olives with similar ripening degree of Picual and Koroneiki varieties grown in Italy and in Spain and Greece, respectively, (Table 2) further confirmed that the volatile composition of oils was an expression of the genetic store of fruits and that climate probably had only an indirect effect by modifying the degree of ripening.

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