Biogeneration of Volatile Compounds in Virgin Olive Oil: Their Evolution in Relation to Malaxation Time

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The volatile composition of oils obtained from fruits of two Italian varieties, Coratina and Frantoio, using different malaxation times of pastes was determined. The results show that the biogeneration of aroma components was very fast and took place just after cell disruption, owing to the activation of enzymes contained in the fruit. The cleavage by heterolytic hydroperoxide lyase is the most important process in both varieties considered and gives rise to C_6 compounds which represent the major compounds of the olive oil aroma. C_6 aldehydes and alcohols increase with the prolonging of malaxation, whereas esters considerably decrease after 30 min. In addition, C_5 alcohols, C_5 carbonyl compounds, and pentene dimers have been detected in the volatile composition of virgin olive oils.

Keywords: *Volatile kinetics; C_6 and C_5 oxygenated bioformation; pentene dimers; olive oil*

INTRODUCTION

In the overall quality of virgin olive oil, the sensory quality plays an important role in directing the preference of consumers. Their volatile composition is tightly related to sensory attributes that can be detected by consumers (Solinas and Angerosa, 1989; Angerosa et al., 1996). Changes in the content of volatile compounds can notably modify the olfactory perceptions.

From a quantitative point of view, the main volatiles are C_6 components that are produced from the so-called lipoxygenase pathway (Hatanaka et al., 1987; Gardner, 1991; Olias et al., 1993). It is generally accepted that lipoxygenases (LOX's) are active on free unsaturated fatty acids; therefore, the triacylglycerols have to be submitted to hydrolysis catalyzed by very active acyl hydrolases (Olias et al., 1993). Moreover, linolenic and linoleic acids are also contained, in suitable quantity, in the lipids of the cellular and subcellular membranes (Alter and Gutfinger, 1982), and a priori the hypothesis that LOX's can act on membrane lipids cannot be rejected. The lipoxygenases, after their release owing to cellular disruption of fruits, immediately become active and transform the unsaturated fatty acids, linolenic (LnA) and linoleic (LA) acids, into their corresponding 9- and 13-hydroperoxides in a ratio ranging between 65:35 and 55:45, respectively (Olias et al., 1993). The absence of C_9 metabolites in the virgin olive oil headspace implies that only the 13-hydroperoxides from LnA (13-HPOT) and LA (13-HPOD) are the substrates for further enzymatic and chemical evolutions.

Once the 13-hydroperoxides are formed, most of them are immediately cleaved to form C_{12} oxo acids and (*Z*)-3-hexenal and hexanal; enzymatic transformations of these aldehydes mediated by isomerases, alcohol dehydrogenases, and esterases yield the corresponding C_6 alcohols and C_6 esters.

During the first steps of oil extraction (crushing and malaxation), considerable changes in the oil chemical composition occur, owing to both the activation of fruit enzymes (Gardner, 1991; Sanz et al., 1992), caused by the disruption of cellular tissues, and the partition phenomena between oil and water and vice versa.

The different kinds of crushers used to produce olive pastes (Angerosa and Solinas, 1990; Angerosa and Di Giacinto, 1995) and the essential malaxation operation (Solinas et al., 1978; Montedoro and Garofolo, 1984; Lanzani et al., 1990; Montedoro, 1992) modify the chlorophyll and phenolic compound contents and are also responsible for the changes of volatiles because of the activation of enzymes involved in the lipoxygenase pathway.

The aim of this research is to gain more information about the technological step considered in determining the volatile production and, possibly, about the pathways involved.

Therefore, the volatiles formed at crushing and at different malaxation times were detected.

Table 1. Ripening Characteristics of Fruits of the Coratina and Frantoio Varieties

	variety	
% fruits with skin	Coratina	Frantoio
green or yellow	24.8	10.1
partial purple	45.8	28.9
total purple or black	29.4	61.0

MATERIALS AND METHODS

Materials. Two Italian varieties, Coratina and Frantoio, were chosen for the experiments.

A homogeneous batch of Coratina or Frantoio fruits, picked by hand at the end of November, with a known ripening degree, was divided into 13 10-kg parcels. The ripening characteristics are summarized in Table 1.

All solvents, for organic residual analysis, were purchased from J.T. Baker (Deventer, Holland); nonan-1-ol (internal standard) was purchased from Aldrich (Steinheim, Germany) and activated charcoal (0.5−0.85 mm; 20−35 mesh ASTM) from E. Merck (Schuchardt, Germany). Charcoal was cleaned by treatment in a Soxhlet apparatus with diethyl ether and tested before the analyses.

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Table 2. Headspace Volatile Compounds (ppm of Nonan-1-ol) of Oils from the Coratina Variety at Different Malaxation Times

	minutes									
ppm nonan-1-ol	0	5	10	15	20	30	45	60	90	120'
ethyl acetate	3.4	5.4	3.6	3.2	4.9	3.5	3.9	4.6	4.3	3.4
methanol	0.5	0.5	0.8	1.1	0.9	0.8	1	1.2	4.4	1.2
2-methylbutanal	1.5	1.4	1.2	1	0.9	2.1	6.1	9.9	8.6	10.4
3-methylbutanal	1.6	1.7	2.1	1.3	1.7	4.1	8.7	14.7	14.8	14.4
ethanol	9.5	17.1	4.8	7.3	8.2	8.2	11.2	11.9	14.6	10
pentene dimers	78.5	54.3	52.5	51.2	48.7	52.2	49.1	53.8	59.2	60.7
pentan-3-one	14.8	9.9	11.4	12.1	11.3	11.4	10.9	9.7	13.7	14.9
1-penten-3-one + unknown compd	41.7	27.1	35.8	42.3	51.1	49.6	53.4	46.4	59.7	48.5
hexanal	9.8	4.9	3.6	5	4.6	4.8	3.7	5.3	6.6	6.9
2-methyl-1-propanol	0.5	1	1.2	1.2	0.8	0.9	0.8	0.9	0.8	1
unknown	1.4	0.8	1.5	1.2	0.8	1	0.9	0.9	1	1
2-pentenal	5.1	3.8	4.5	5.4	6.1	6.1	6.8	6.5	6.8	7.7
1-penten-3-ol	32.5	19.5	23.1	35	38.4	40	38	39.6	55.4	49.2
2-methylbutan-1-ol + 3-methylbutan-1-ol	7.9	7.3	6.5	5.9	6.5	6.4	6.4	7.4	7.1	7.3
trans-2-hexenal	601.8	299.4	324.5	314.9	446.9	438.4	374.4	458.2	521.8	559.5
pentan-1-ol	3.1	1.1	1	0.5	0.4	0.4	0.4	0.4	0.4	0.5
hexyl acetate	1.6	1.1	1.1	1.1	1	1.2	0.7	0.5	0.5	0.2
trans-2-penten-1-ol	1.9	1.1	1.3	1.5	2	2.4	2.1	2.1	3	2.5
cis-3-hexenyl acetate	12.6	5.1	8.6	9.7	10.3	9.3	5	2.7	3.7	1
cis-2-penten-1-ol	24.2	13.4	14.9	15.8	24.8	25.5	24.1	25.6	34.3	32.1
hexan-1-ol	5.4	4.4	4	4.2	4.7	5.8	5.6	6	6.9	7.1
cis-3-hexen-1-ol	8.5	4.9	4.8	5.4	6.9	7.8	7.4	6.9	12.1	11.9
trans-2-hexen-1-ol	6	5.5	4.5	4.6	4.4	5.4	5.5	7.2	10.3	10.3
acetic acid	4.5	3.6	4	3.8	2.3	3.9	2.4	2.6	2.5	2.1
copaene	7.6	7.7	7.3	6.7	6.9	8.8	8.4	8.7	9.5	8.6
propanoic acid	0	0	0	0	0	0	0	0	0.4	0.3

Methods. Oil was extracted as follows:

(1) Crushing of olives was performed using an inox hammer mill operating at 3000 rpm and provided with a sieve with 5-mm holes.

(2) Malaxation of pastes was made in a mixer at 50 rpm at 25 °C. The malaxation times selected were the following: 0, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min. Water (2860 mL) was added immediately after crushing in the cases of the 0-, 5-, and 10-min malaxation time experiments and 10 min before the end of malaxation operation in the remaining cases.

(3) Centrifugation of the paste was performed using a basket centrifuge at 1400*g* for 1 min in order to remove the solid remains.

The resulting liquid phase was put into a cylinder and allowed to decant for 30 min. The upper oil layer was centrifuged for 5 min at 3000*g*.

The enzymatic inactivation was performed by heat-plunging parcels of 10 kg of olives each one into 15 L of water holding the water at the boiling temperature for 1, 3, and 5 min, respectively.

Volatile compound extraction was carried out according to the procedure previously described (Angerosa et al., 1990)

GC Analysis. Gas chromatography was carried out with a Carlo Erba Mega Series 5160 fitted with a Nordion silica capillary Carbowax 20 M column (50 m length; 0.32 mm i.d.; 0.5 mm 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 the following: isotherm at 25 °C for 7 min, from 25 to 33 °C at 0.8 °C min^{-1}, from 33 to 80 °C at 2.4 °C min^{-1}, and from 80 to 155 °C at 3.7 °C min^{-1}; final isotherm at 155 °C for 20 min. The temperature of the detector was held at 240 °C; the carrier gas was H_2 at 30 kPa. The injection volume was 0.5 mL. Quantitation was achieved by peak area integration with a Carlo Erba Mega Series integrator.

RESULTS AND DISCUSSION

DHS-GC analysis of oils obtained from olives after enzyme inactivation showed a significant decrease of concentration of volatile compounds in comparison with that of an oil extracted from fresh fruits in the same operative conditions, when the heating was provided for 1 min. By increasing the time of heating (3 min), the inactivation was more effective. The low quantities of C_6 aldehydes, C_6 alcohols, and C_5 ketones, in addition to the peaks of acetone, ethyl acetate, and ethanol, detected in the oil derived from olives heated for 3 min (Figure 1), can be explained by the fact that the kernel was not completely submitted to enzyme inactivation since the time of the experiment was not long enough. It was not possible to test the effectiveness of heating for a longer time (5 min) since the oil could not be extracted from pastes because a firm emulsion between oil and the water/solid system was always observed. The results of these experiments allowed us to conclude that most of volatiles are formed through the action of endogenous enzymes contained in the fruit (Gardner, 1991; Sanz et al., 1992).

The concentrations of the volatile compounds at the different malaxation times are reported in Tables 2 and 3 for the oils obtained from the Coratina and Frantoio varieties, respectively. The reported values are the mean values calculated from three independent experiments; the confidence limits were always below 10%. Thirty-three were the identified compounds. Among them, ethyl acetate, methanol, 2-methylbutanal, 3-methylbutanal, ethanol, 2-methylbutan-1-ol + 3-methylbutan-1-ol, butan-1-ol, acetic acid, and propanoic acid arose from the degradation of some amino acids (valine, leucine, and isoleucine) and from the fermentation of sugars naturally occurring in the fruits; the others were C_5 and C_6 components derived from the enzymatic transformation of unsaturated fatty acids.

It must be pointed out that the volatile concentration at zero minutes of malaxation time is high. This means that their biogeneration is very fast and takes place just after cell disruption. The contents of a large part of the volatile compounds sharply decrease in the oils obtained after 5 min of olive paste malaxation; from this time

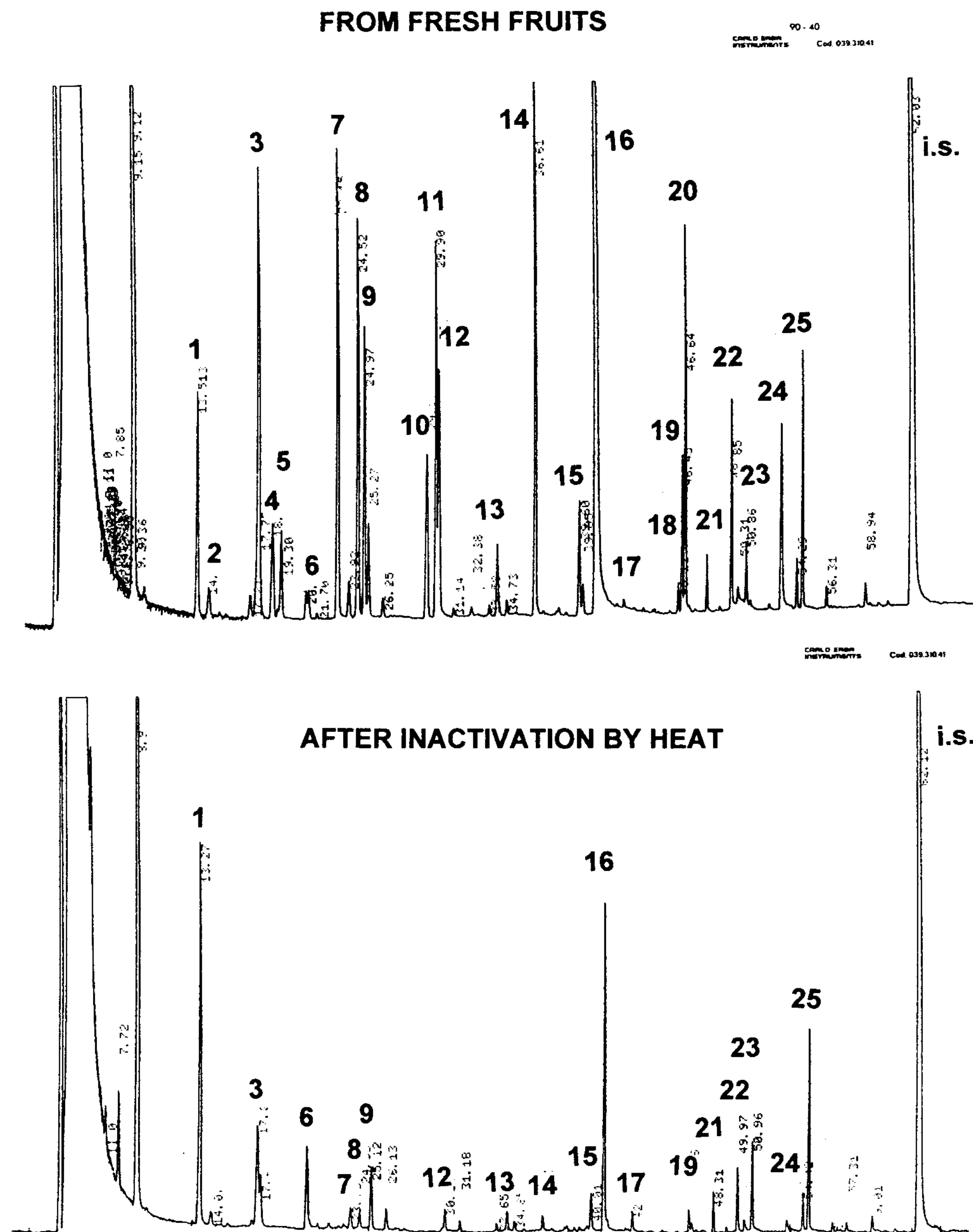

Figure 1. Gas chromatograms of volatile compounds of oils from fresh fruits and after 3 min of enzyme inactivation by heat. 1 = ethyl acetate; 2 = methanol; 3 = ethanol; 4 = pentene dimer; 5 = pentene dimer; 6 = pentan-3-one; 7 = pentene dimer; 8 = pentene dimer; 9 = 1-penten-3-one; 10 = pentene dimer; 11 = pentene dimer; 12 = hexanal + pentene dimer; 13 = 2-pentenal; 14 = 1-penten-3-ol; 15 = 2-methyl- + 3-methylbutan-1-ol; 16 = *trans*-2-hexenal; 17 = pentan-1-ol; 18 = *trans*-2-penten-1-ol; 19 = *cis*-3-hexenyl acetate; 20 = *cis*-2-penten-1-ol; 21 = hexan-1-ol; 22 = *cis*-3-hexen-1-ol; 23 = *trans*-2-hexen-1-ol; 24 = acetic acid; 25 = copaene; i.s. = internal standard (nonan-1-ol). Furthermore, in other chromatograms, the following compounds were also identified: acetone (RT = 9.5); 2-methylbutanal (RT = 14.6); 3-methylbutanal (RT = 15.1); ethyl propionate (RT = 17.2); 2-methylpropan-1-ol (RT = 31.2); butan-1-ol (RT = 32.6); hexyl acetate (RT = 44.2); propanoic acid (RT = 63.5).

onward, they begin, more or less slowly, to increase with the prolonging of malaxation.

These findings could be explained as follows. The minute oil droplets formed during the olive fruit crushing are dipped in the vegetation water, rich in proteins and phospholipids (Martinez Moreno et al., 1957), and therefore, they are surrounded by lipoproteic membranes that prevent the exchange of metabolites between the oily and water phases. During the first 5 min of paste kneading, a great quantity of minute oil droplets coalesces in drops with a diameter greater than 30 μm, which can be easily extracted (Martinez Moreno et al., 1957). The coalescence phenomenon, promoted by the slow mixing of pastes, implies repeated cleavages of lipoproteic membranes, so allowing the ripartition of compounds between oil and water. Since in the first 5 min the lipoproteic membranes of the small oil droplets are not broken enough, the exchange of metabolites is quite low. At this point, the increased oil recovery (from 1.4 to 3.1% for Coratina and from 2.2 to 3.6% for Frantoio) causes a real dilution of volatiles formed during the crushing. From this time on, there would be competition among some factors, such as greater oil recovery, volatile production (much more slowly than at crushing), and, above all, partition phenomena of compounds between the oil and water and vice versa.

The latter phenomena only begin with the malaxation of pastes, so the trend of the production of the different volatile compounds must be followed in the experiments carried out on malaxed pastes.

Table 3. Headspace Volatile Compounds (ppm of Nonan-1-ol) of Oils from the Frantoio Variety at Different Malaxation Times

	minutes									
ppm nonan-1-ol	0	5	10	15	20	30	45	60	90	120
ethyl acetate	0.6	1.3	1.9	2.1	2.6	3.4	4.3	4.2	4.6	5.8
methanol	1.9	2.1	1.3	0.2	0.1	0.3	0.4	0.5	1.5	2.9
2-methylbutanal	0.1	0.1	0.1	0.8	0.8	1.8	2.3	8.5	7.4	4.1
3-methylbutanal	0.1	0.1	0.1	1.1	1.1	3.1	5	12.1	14.9	10
ethanol	7.8	9.3	8.6	5.4	2.6	3.2	2.9	3.8	4.2	4.2
pentene dimers	25.7	32	42.7	40.4	47.5	46.2	51.4	50	47.4	51.5
pentan-3-one	2.4	2.8	4.5	12.2	10	11.6	14.8	14	17	17.3
1-penten-3-one + unknown compd	16.9	21.3	41.8	43.2	56.1	57.5	62.5	66	61.7	68.1
hexanal	0.3	8.1	12	12.1	20.3	31	34.7	58.3	133.4	222.5
2-methyl-1-propanol	0.2	0.2	0.2	0.3	0.3	0.4	0.5	0.5	0.6	0.6
unknown	0	0	0	1.2	0.8	1	1.4	1.8	1.1	1.3
2-pentenal	3.3	4.3	5	7.6	7.9	8.7	10.9	10	12	13.4
1-penten-3-ol	10.2	11.9	17.1	24.9	28.7	31	41.3	42	51.4	61.2
2-methylbutan-1-ol + 3-methylbutan-1- ol	3.8	4.6	6.5	7.6	7.4	6.7	7.7	10.4	10.4	11.1
trans-2-hexenal	764.9	485.6	691.5	759.6	1078.1	960.5	1312.5	1476.8	1522.6	1573.6
pentan-1-ol	0.1	0.1	0.1	0.5	0.1	0.1	0.3	0.7	0.7	0.8
hexyl acetate	3.5	2.5	3.9	3.9	3.7	3.6	3.4	3.1	2.6	2.7
trans-2-penten-1-ol	0.6	0.7	0.7	1.8	1.9	2.3	2.6	2.5	2.9	3.5
cis-3-hexenyl acetate	20	13.8	20.8	19.2	16.4	16.8	15.6	9.1	8.3	12
cis-2-penten-1-ol	0.3	1.1	1.2	15.7	20.9	21.7	30	27.6	42.5	42.6
hexan-1-ol	5.7	1.9	4.5	8.4	8.5	7.4	9.1	7	6.3	12.5
cis-3-hexen-1-ol	5.7	4.9	6.5	11.9	11.7	8.8	11.9	12	13	13.7
trans-2-hexen-1-ol	4.1	3.8	6.6	8.2	9.5	12.4	14.9	14.7	21.5	29.1
acetic acid	0.1	0.1	0.6	0.6	1.5	2.7	2.7	2.9	2.7	3.6
copaene	0.1	0.1	0.1	0.5	0.5	0.5	0.4	0.4	0.5	0.5
propanoic acid	0	0	0	0	0	0	0	0	0.7	0.7

Table 4. Total Amount (ppm of Nonan-1-ol) of C_5 and C_6 Compounds Having Linolenic and Linoleic Acids as Precursors

	minutes									
	0	5	10	15	20	30	45	60	90	120
	Coratina Variety									
C_6 compds from LnA	628.9	314.9	342.4	334.6	468.5	460.9	392.3	475.0	547.9	582.7
C_6 compds from LA	16.8	10.4	8.7	10.3	10.3	11.8	10.0	11.8	14.0	14.2
C_6 from LnA/C_6 from LA	37.4	30.3	39.4	32.5	45.5	39.1	39.2	40.3	39.1	41.0
pentene dimers	78.5	54.3	52.5	51.2	48.7	52.2	49.1	53.8	59.2	60.7
C_5 alcohols from LnA	58.6	34.0	39.3	52.3	65.2	67.9	64.2	67.3	92.7	83.8
C_5 carbonyl compds from LnA	48.6	30.9	40.3	47.7	57.2	55.7	60.2	52.9	66.3	55.4
pentenols + C_5 carbonyl compds both from LnA	105.4	64.9	79.6	100.0	122.4	123.6	124.4	120.2	159.0	139.2
C_5 + C_6 compds from LnA	814.6	434.1	427.5	485.8	639.6	636.7	565.8	649.0	766.1	782.6
% C_6 compds from LnA/C_5 + C_6 compds from LnA	77.2	72.5	80.1	68.9	73.2	72.4	69.3	73.2	71.5	74.5
	Frantoio Variety									
C_6 compds from LnA	794.7	508.1	725.4	798.9	1115.7	998.5	1354.9	1512.6	1565.4	1628.4
C_6 compds from LA	9.5	12.5	20.4	24.4	32.5	42.0	47.2	68.4	142.3	237.7
C_6 from LnA/C_6 from LA	83.7	40.6	35.6	32.7	34.3	23.8	28.7	11.1	11.0	6.9
pentene dimers	25.7	32.0	42.7	40.4	47.5	46.2	51.4	50.0	47.4	51.5
C_5 alcohols from LnA	11.1	13.7	19.0	42.4	51.5	55.0	73.9	72.1	96.8	107.3
C_5 carbonyl comds from LnA	20.2	25.6	46.8	50.8	64.0	66.2	73.4	76.0	73.7	81.5
pentenols + C_5 carbonyl compds both from LnA	31.3	39.3	65.8	93.2	115.5	121.2	147.3	148.1	170.5	188.8
C_5 + C_6 compds from LnA	851.7	579.4	833.9	932.5	1279.0	1165.9	1553.7	1711.1	1782.9	1868.3
% C_6 comps from LnA/C_5 + C_6 comps from LnA	93.3	87.7	87.0	85.7	87.3	85.6	87.2	88.4	87.8	87.1

The major components of the volatile fraction of oils from the Coratina and Frantoio varieties were C_6 compounds, in particular *trans*-2-hexenal, arising from the heterolytic cleavage of the 13-hydroperoxides of LnA and LA by enzymes involved in the hydroperoxide lyase cascade. The amount of compounds arising from LnA is always really greater than that of compounds from LA according to literature data (Olias et al., 1993). C_6 alcohols increase during the experiments, whereas esters, especially *cis*-3-hexenyl acetate, undergo a considerable decrease after 30 min of malaxation, probably due to a progressive inactivation of esterases (Tables 2 and 3).

Besides in the aromas of the examined virgin olive oil samples, quite large quantities of C_5 compounds were detected. These compounds were pentenols, 2-pentenal, and 1-penten-3-one, identified by comparison of their mass spectra with those of reference compounds, and seven pentene dimers, recently fully characterized by GC−MS−CI (Angerosa et al., 1998). In particular, the structures of the pentene dimers imply coupling reactions of 1−3 pentene allylic radicals. Really the number of pentene dimers detected in olive oils is exactly that of all possible coupling combinations of these radicals. Intermediates such as 1−3 pentene allylic radicals have been described in the homolytic cleavage pathway of the 13-hydroperoxides to give C_5 compounds. The 13-hydroperoxides could be submitted to a enzyme-mediated cleavage, passing through an alkoxyl radical which subsequently undergoes β-scission, giving rise to a pentene allylic radical. This could form pentenols, subtracting hydroxy radicals from the medium, or pentene dimers owing to coupling of two allylic radicals (Salch et al., 1995). The pentenols could be oxidated and C_5 carbonyl compounds produced (Gardner et al., 1996).

The presence of all possible C_5 compounds attributable to a β-scission mechanism allows us to suppose, with a reasonable degree of probability, that the 13-alkoxy radical of LnA is an intermediate involved in the aroma bioformation in olive pulp, like in soja seeds (Salch et al., 1995).

Pentene dimers show contents practically constant in all experiments in the Coratina variety, while in the Frantoio they increase (Table 4). Pentenols and C_5 carbonyl compounds increase with the malaxation time in both varieties. This finding can be partially explained by the different solubilities for the pentene dimers and the other C_5 components. In fact, hydrocarbons solubilize faster that oxygenated compounds in the oily matrix, reaching a faster ripartition equilibrium. However, in both cultivars, a careful analysis of our experimental data and, especially, the behavior of *cis*-2-penten-1-ol compared with that of 1-penten-3-ol, induced us to believe that other pathways different from the β-scission mechanism could be present which could contribute to the C_5 oxygenated compound bioformation. In fact, recently in other substrates, a homolytic hydroperoxide lyase catalyzing the specific cleavage of 13-HPOT to form 13-oxo acid and 2-penten-1-ol has been found (Kondo et al., 1995).

The cleavage by heterolytic hydroperoxide lyases is the most important process in both varieties considered; in fact, the total amount of C_6 components from LnA ranges between 72 and 87% of all compounds having LnA as the precursor in all experiments performed (Table 4). Unsaturated C_5 alcohols and C_5 carbonyl compounds are the main volatile metabolites produced by the β-scission mechanism and the other possible pathway (Table 4).

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