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Influence of malaxation temperature and time on the quality of virgin olive oils

Franca Angerosa *, Roberta Mostallino, Carla Basti, Raffaella Vito

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

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Abstract

To study the influence of operative conditions adopted during the malaxation of pastes on the quality of resulting oils, we compared sensory characteristics, secoiridoid compounds and the volatile composition of oils extracted from homogeneous batches of olive fruits from Coratina and Frantoio cultivars by using different malaxation times and temperatures. Malaxation time, and especially temperature, negatively affected the intensity of sensory attributes and the content of secoiridoid compounds, modified the composition of metabolites arising from lipoxygenase (LOX) pathways, reducing volatile compounds displaying pleasant sensations and increasing those giving less attractive perceptions, and also elevated the production of 2-methyl butanal and 3-methyl butanal through amino acid conversion. Low temperatures and times, ranging between 30 and 45 min, according to the rheology of the olive pastes, were the optimal operative conditions for the malaxation.

Keywords: Olive oil; Volatile compounds; Malaxation conditions; Sensory attributes

1. Introduction

Virgin olive oils are extracted from fruits of *Olea europaea* L. by using only physical methods, which include crushing of olives, malaxation of resulting pastes and separation of the oily phase. All operations involved in the oil extraction process are directed towards obtaining the highest quantity of oil from fruits. The malaxation step, consisting of a low and continuous kneading of olive pastes, is especially useful for achieving high and satisfactory yields of extraction. In fact this essential technological operation helps the small droplets of the oil formed during the milling, in particular by means of metallic crushers, to merge into large drops that can be easily separated through mechanical systems, and breaks up the oil/water emulsions.

Because of its location in mesocarp of cells and the use of purely mechanical pieces of apparatus for its extraction, virgin olive oil does not require further treatments before its consumption. Therefore, it preserves a great number of volatile and nonvolatile compounds responsible for its fragrant and peculiar flavour.

Phenolic compounds are contained in the endocellular oil; malaxation is the step of the oil extraction that especially modifies their qualitative and quantitative composition (Montedoro, Baldioli & Servili, 1992; Servili, Baldioli & Montedoro, 1992). Phenolics are mainly responsible for the shelf-life of the oils (Baldioli, Servili, Perretti & Montedoro, 1996; Papadopoulos & Boskou, 1991) and also for their typical bitter taste (Montedoro et al., 1992).

Among volatile compounds present in virgin olive oil aromas, C_6 compounds, in particular C_6 aldehydes, show the higher concentrations. C_6 volatile compounds, which are common contributors of the aroma of many fruits and vegetables, are enzymatically produced from polyunsaturated fatty acids containing a *cis-cis* 1,4 pentadiene structure through the so-called lipoxygenase (LOX) pathway (Hatanaka, 1993; Vick & Zimmerman, 1987). Many enzymes are involved in this pathway and some of them were recently isolated and characterized in pulp tissues of olive fruits (Olías, Perez, Rios & Sanz, 1993; Salas & Sánchez, 1998a,b,c). According to this pathway, the oxidation, catalyzed by LOX, of linoleic (LA) and linolenic (LnA) acids to 9- and 13-hydroperoxides is followed by the cleavage,

* Corresponding author. Fax: +39-085-959518.
E-mail address: elaiotec@unich.it (F. Angerosa).

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mediated by hydroperoxide lyase (HPL), of only 13-hydroperoxides because of the high substrate specificity of this enzyme (Olías et al.; Salas & Sánchez 1998a). The products of this enzymatic cleavage are C_6 aldehydes. Alcohol deydrogenase (ADH) is involved in the reduction of C_6 aldehydes and alcohol acetyl transferase (AAT) in the enzymatic esterification of C_6 alcohols.

On the other hand, the head-space of virgin olive oils shows quite a large concentration of C_5 compounds, in addition to C_6 volatile metabolites. Investigations, carried out in recent years, gave evidence that the production of C_5 compounds could be explained in virgin olive oils as a result of the activity of an additional branch of the LOX pathway (Angerosa, d'Alessandro, Basti & Vito, 1998; Angerosa, Camera, d'Alessandro & Mellerio, 1998), already proved in soy seeds (Salch, Grove, Tukamura & Gardner, 1995).

The LOX pathways become active at the olive crushing and C_6 and C_5 volatile compounds, because of their solubility, are quickly incorporated in the oily phase and accumulate during the malaxation of the resulting pastes.

C_5 and especially C_6 components contribute to green odour notes (Guth & Grosh, 1991, 1993; Morales, Angerosa & Aparicio, 1999) and consequently changes of the concentrations of each C_6 and C_5 compound can notably modify the sensory perceptions.

A previous work gave evidence that the concentrations of C_6 and C_5 volatile metabolites arising from LOX pathways, change in relation to malaxation time (Angerosa, d'Alessandro et al., 1998). On the other hand, the activity of enzymes involved in the LOX pathways is affected in a different way by the temperature of the olive paste during the malaxation (Salas & Sánchez, 1999). Therefore, it was thought interesting to study the influence of condition of paste malaxation on the quality of virgin olive oils.

The virgin olive oil quality is also inversely related to the amount of some compounds whose production is closely connected with degradation phenomena of the raw material. Such processes start when there is contact between pulp tissues and some micro-organisms naturally occurring on the cuticle of fruits (Angerosa, Lanza & Marsilio, 1996). The fermentation of sugars contained in the fruit, the enzymatic transformation of ethanol into acetic acid, and in particular, the conversion of valine, leucine and isoleucine into branched aldehydes, branched alcohols and their corresponding acids (Tressl & Drawert, 1973; Tressl & Jennings, 1972), are promoted by contact time and temperature. This suggests that further information about oil quality could be obtained by monitoring changes of compounds from the aminoacid conversion with respect to the conditions adopted during the malaxation of olive pastes.

2. Materials and methods

2.1. Samples

Homogeneous batches (100 kg each) of olive fruits (*Olea europaea L.*) from the Italian Coratina and Frantoio cvs, picked by hand at a known ripening degree were divided into ten lots. A laboratory mill, equipped with a metal crusher, a mixer and a basket centrifuge, were used to extract the oil from the fruits. Ten samples of each cv were extracted from each homogeneous batch using different malaxation times (15, 30, 45, 60 and 90 min) and temperatures (25 and 35°C). Unfortunately the processing of fruits from Coratina cv at 35°C and 45 min of paste malaxation could not be carried out because of technical troubles.

2.2. Chemicals

All solvents, for organic residual analysis, were purchased from J. T. Baker (Deventer, Holland); resorcin, bis(trimethylsilyl)trifluoracetamide (BSTFA), *trans*-2-hexenal, hexyl acetate, hexan-1-ol and activated charcoal (0.5–0.85 mm; 20–35 mesh ASTM) were 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, *trans*-2-penten-1-ol, 2-methyl butanal and 3-methyl butanal 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, 2-methyl butan-1-ol, 3-methyl butan-1-ol, 2-methyl propan-1-ol, and propanoic acid from Fluka Co. (Buchs, Switzerland). Pentene dimers were synthesized from *trans*-3-hexenoic acid according to a previous paper (Angerosa, Camera et al., 1998).

2.3. Sensory analysis

The sensory evaluation of olive oils was performed according to Annex XII of EC regulation n. 2568/91. However, the number of descriptors of the official profile sheet was increased to allow a more careful description of "green" perceptions. With the aim of dissecting and describing different shades of green perceptions, eight fully trained tasters were requested to assess oil samples extracted from unripe fruits of different cultivars and to describe their perceptions freely by means of their own associations with sensations perceived during former experiences. After a consensus-building discussion held with assessors to remove possible semantic differences, the descriptors most commonly used were introduced on the profile sheet. Fourteen sensory attributes were considered: fruity (green olives), bitter, pungent, green cut lawn, leaf, apple, wild

flower, artichoke, walnut husk, green tomato, almond, green hay, butter/cream, green banana. The intensity scale ranged from zero to 5. The sample presentation was fully randomized and oils were evaluated in triplicate.

2.4. Volatile extraction and GC analysis

Volatile compound extraction was carried out according to a dynamic head-space technique previously described (Angerosa, Giacinto & d'Alessandro, 1997).

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 was at 155°C for 20 min. The temperature of the detector was held at 240°C; carrier gas was H_2 at 30 kPa. Injection volume was 0.5 ml.

2.5. 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 compounds were synthesized from *trans*-3-hexanoic acid and their mass spectra were obtained (Angerosa, Camera et al., 1998).

2.6. Quantitation

Each C_5 and C_6 compound was quantified on the basis of its gas chromatographic areas using its calibration curve previously drafted (Angerosa et al., 1997; Angerosa, Camera et al., 1998; Angerosa, d'Alessandro, Di Girolamo, Vito & Serraiocco, 1999). Such curves were obtained by adding known quantities of C_5 and C_6 compounds to a recently refined olive oil. The amounts of C_6 and C_5 compounds, expressed as ppm, were the mean values calculated from three independent experiments; the confidence limits were always below 10%. Some of the remaining compounds of olive oil aroma, not deriving from the LOX pathway, reported in Fig. 1, were quantified as ppm of nonan-1-ol (internal standard).

2.7. Evaluation of phenolic compounds

Extraction and purification of phenolic compounds were performed using 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 submitted to GC-MS analysis (Angerosa, d'Alessandro, Konstantinou & Di Giacinto, 1995). The amounts of secoiridoid compounds were expressed as ppm of resorcin (internal standard).

2.8. 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

Previous researches proved that malaxation conditions affected the flavour of the resulting oil, involving changes of concentration of both polyphenols (Servili, Baldioli, Selvaggini, Mariotti, Federici & Montedoro, 1998) and volatile compounds (Angerosa, d'Alessandro et al., 1998; Kiritsakis, 1998).

It is accepted that C_6 and C_5 metabolites are major contributors to green sensory perceptions (Morales et al. 1999), whereas the polyphenolic fraction is considered to be mainly responsible for bitter (Gutierrez Rosales, Perdiguero, Guttierrez & Olías, 1992) and pungent perceptions (Withehead, Breeman & Kinsella, 1985).

To better understand the changes in flavour due to operative conditions adopted in the experiments performed, the total amount of secoiridoid compounds and the volatile composition of the oils extracted from fruits of two Italian cvs (Coratina and Frantoio, respectively) were compared in relation to different times and temperatures of olive paste malaxation, respectively. Experiments were planned by using homogeneous batches of fruits and the same processing plant, so that any modifications of flavour, and consequently of concentrations of both secoiridoid and volatile compounds, had to be exclusively attributed to different operative conditions of malaxation.

Table 1 summarizes the trend of secoiridoid compounds with respect to time and temperature of paste malaxation. Total amount of the secoiridoid fraction was negatively affected by malaxation time. Similar results had been obtained by Servili et al. (1998) who attributed the reduction of phenolic concentration to the activation of endogenous oxidoreductase enzymes. However, the greater losses in our experiments were found when temperature was raised from 25 to 35°C, in agreement with data already published (Servili, Baldioli

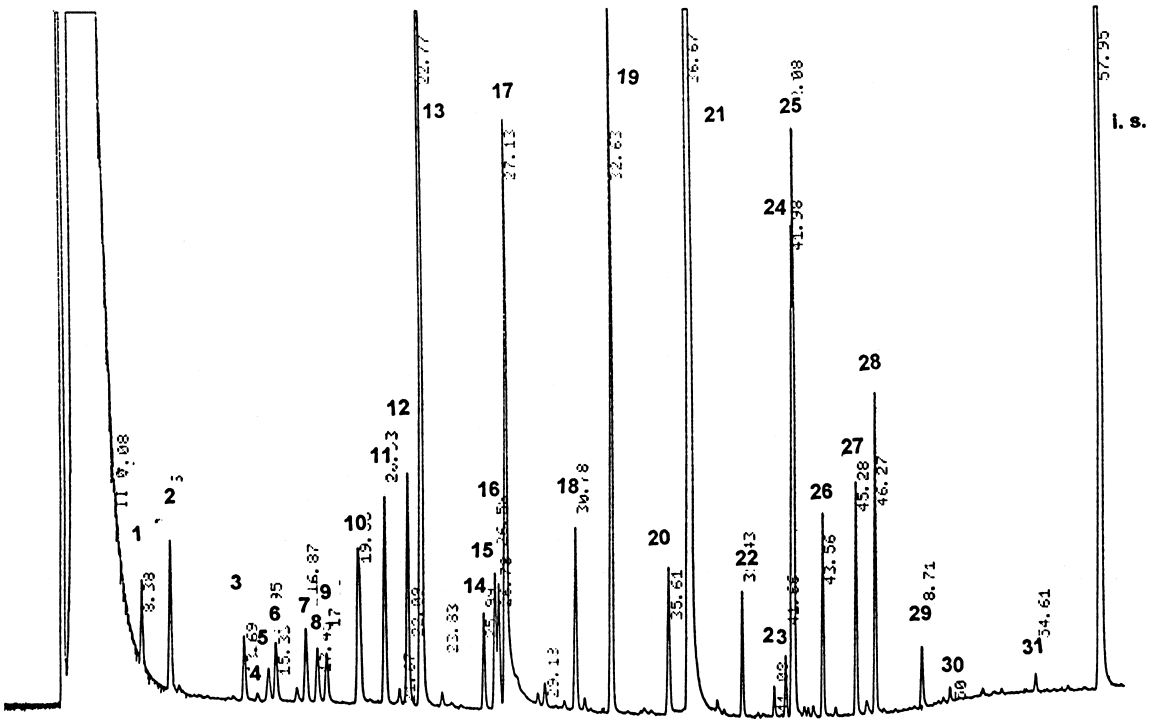

Fig. 1. Dynamic head-space gas chromatogram of volatile components of oil extracted from Frantoio olives using malaxation at 25°C from 30 min. The volatile fraction was trapped on charcoal and desorbed with diethyl ether prior to the GC analysis. Peaks: 1, *n*-octane; 2, acetone; 3, ethyl acetate; 4, methanol; 5, 2-methyl butanal; 6, 3-methyl butanal; 7, ethanol; 8, pentane dimer; 9, pentene dimer; 10, pentan-3-one; 11, pentene dimer; 12, pentene dimer; 13, 1-penten-3-one; 14, pentene dimer; 15, pentene dimer; 16, pentene dimer; 17, hexanal; 18, *trans*-2-pentanal; 19, 1-penten-3-ol; 20, 2-methyl butan-1-ol + 3-methyl butan-1-ol; 21, *trans*-2-hexanal; 22, hexyl acetate; 23, *trans*-2-penten-1-ol; 24, *cis*-3-hexenyl acetata; 25, *cis*-2-penten-1-ol; 26, hexan-1-ol; 27, *cis*-3-hexen-1-ol; 28, *trans*-2-hexen-1-ol; 29, acetic acid; 30, copaene; 31, octan-1-ol, i.s., nonan-1-ol (internal standard).

& Montedoro, 1994), that showed that malaxation temperature caused a marked decrease in concentration, in particular of derivatives containing hydroxytyrosol (3,4-dihydroxyphenylethanol).

The volatile fraction of oils obtained by using different malaxation conditions was mainly formed of C_6 and C_5 compounds, produced from LnA and LA through the LOX pathways. Their quantitative composition basically changed according to the genetic store of cultivars (Angerosa, Basti & Vito, 1999). However, some modifications of the concentration of C_6 and C_5 compounds from the LOX pathways should be attributed to changes of operative conditions adopted during the malaxation step of the oil extraction process (Table 2).

According to previous research (Angerosa, d'Alessandro et al., 1998), this investigation confirms that (i) the cleavage by heterolytic HPL was the most important process because of higher amounts of C_6 compounds than C_5 metabolites and (ii) there was an accumulation of C_6 and C_5 volatile compounds with the prolonging of malaxation time, regardless of the temperature adopted. Data again proved that C_6 and C_5 alcohols and carbonyl compounds, in particular *trans*-2-hexenal and hexanal, increased with the malaxation time, whereas a considerable decrease was detected for C_6 esters, especially for *cis*-3-hexenyl acetate, from 30 min on.

In addition, Table 2 shows that the malaxation temperature generally causes a decrease of levels of volatile compounds from LOX pathways, as a consequence of proved inactivation of hydroperoxide lyases (Salas & Sánchez, 1999).

In particular, the malaxation temperature produced both a very considerable loss of C_6 esters, *cis*-3-hexen-1-ol and C_5 metabolites and an increase in hexan-1-ol and

Table 1
Total amount of secoiridoid compounds of oils extracted at the indicated malaxation times and temperatures (as ppm resorcin)

	15′		30′		45′		60′		90′	
Cultivar	25°C	35°C	25°C	35°C	25°C	35°C	25°C	35°C	25°C	35°C
Coratina	651	357	511	357	470		426	263	363	219
Frantoio	179	105	93	87	78	71	61	30	31	27

Table 2
Amounts of C_6 and C_5 compounds from LOX pathways, expressed as ppm, in oils obtained from two Italian cvs by using different times and temperatures of malaxation of olive pastes

	25°C					35°C				
	15′	30′	45′	60′	90′	15′	30′	45′	60′	90′
Coratina cv										
Hexanal	1.1	1.2	1.0	1.3	1.6	0.3	0.6		0.9	1.5
Hexan-1-ol	0.2	0.2	0.2	0.2	0.3	0.1	0.1		0.2	0.3
Hexyl acetate	0.1	0.1	0.0	0.0	0.0	0.0	0.0		0.0	0.0
Trans-2-hexanal	12.6	18.3	15.2	18.7	21.2	15.9	20.8		26.5	33.4
Trans-2-hexen-1-ol	0.2	0.2	0.3	0.4	0.5	0.3	0.4		0.5	0.9
Cis-3-hexen-1-ol	0.2	0.3	0.3	0.3	0.4	0.2	0.2		0.2	0.3
Cis-3-hexenyl acetate	0.8	0.8	0.4	0.2	0.3	0.2	0.2		0.1	0.1
Pentene dimers	0.7	0.6	0.7	0.8	0.8	0.6	0.7		0.6	0.7
1-Penten-3-one	0.6	0.8	0.8	0.7	0.9	0.5	0.4		0.4	0.4
Trans-2-pentenal	0.1	0.1	0.2	0.2	0.2	0.1	0.2		0.1	0.1
1-Penten-3-ol	0.4	0.5	0.5	0.5	0.7	0.3	0.5		0.5	0.7
Trans-2-penten-1-ol	0.0	0.0	0.1	0.1	0.1	0.1	0.1		0.1	0.1
Cis-2-penten-1-ol	0.5	0.6	0.7	0.7	1.0	0.5	0.7		0.7	0.9
Frantoio cv										
Hexanal	0.7	1.7	1.6	2.2	4.3	1.3	1.0	1.7	2.2	4.5
Hexan-1-ol	0.4	0.4	0.5	0.2	0.3	0.1	0.1	0.1	0.2	0.2
Hexyl acetate	0.3	0.3	0.3	0.3	0.2	0.0	0.1	0.1	0.1	0.1
Trans-2-hexenal	30.9	39.1	53.4	60.0	61.9	27.8	30.3	45.3	39.4	49.7
Trans-2-hexen-1-ol	0.4	0.6	0.7	0.7	1.0	0.4	0.4	0.7	0.8	1.6
Cis-3-hexen-1-ol	0.5	0.3	0.4	0.3	0.5	0.1	0.1	0.2	0.2	0.2
Cis-3-hexenyl acetate	1.5	1.3	1.2	0.7	0.7	0.3	0.2	0.2	0.2	0.2
Pentene dimers	0.6	0.6	0.7	0.7	0.7	0.5	0.4	0.6	0.6	0.6
1-Penten-3-one	0.6	0.9	1.0	0.9	0.9	0.5	0.6	0.6	0.6	0.4
Trans-2-pentenal	0.2	0.2	0.3	0.3	0.3	0.1	0.2	0.2	0.2	0.2
1-Penten-3-ol	0.3	0.4	0.5	0.5	0.6	0.2	0.3	0.4	0.4	0.5
Trans-2-penten-1-ol	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1
Cis-2-penten-1-ol	0.5	0.6	0.8	0.8	1.2	0.3	0.5	0.6	0.6	0.8

trans-2-hexen-1-ol. Research developed by Aparicio and his group (Aparicio, Alonso, Morales & Calvente, 1994; Aparicio, Morales & Alonso, 1996) related hexan-1-ol to a "rough" sensation and *trans*-2-hexen-1-ol to perceptions not completely appreciated by most consumers. These results were in agreement with the different odour characteristics found by Bedukian (1971) for *cis* and *trans* forms of primary hexenols, in which tasters judged the *cis* forms to be more attractive than the *trans*.

The amount of *trans*-2-hexen-1-ol was approximately twice as high in Frantoio cv, as in the Coratina variety, depending on the genetic levels of alcohol dehydrogenases of the two cultivars (Angerosa, Basti et al., 1999). In any case the increase of *trans*-2-hexen-1-ol, due to the malaxation temperature, was more steady in oils of the Coratina variety than in those from Frantoio cv and very similar at 90 min.

By evaluating the effect of time and temperature of malaxation on volatile composition, it could be observed that the production of hexanal, one of the most important contributors to the olive oil flavour because of its low odour threshold (Grosh, 1994), seemed mainly promoted by the prolonging of malaxation time; temperature changes of pastes could not produce important effects. Conversely the formation of both C_6 esters from LA and LnA, that contribute to the elicitation of pleasant fruity perceptions (Grosh, 1993), was negatively affected by temperature more than malaxation time. The increase of malaxation temperature and, only in a reduced proportion, the malaxation time, caused the increase of *trans*-2-hexen-1-ol (Table 2).

The concentrations of C_5 compounds were influenced more by temperature adopted during the malaxation than the time (Table 2). The most important losses were detected for 1-penten-3-one, which in previous research was found to be related to bitter and pungent sensations (Angerosa, Mostallino, Basti & Vito, 2000). The relationships between sensory attributes and secoiridoid and volatile compounds from LOX pathways is summarized in Table 3.

On the basis of changes in secoiridoid and volatile compounds, produced at different malaxation times and temperatures, the sensory modifications that could be expected were a weakening of bitter, pungent and leaf attributes in oils obtained at higher temperatures and

Table 3
Relationships between sensory attributes and both secoiridoid and volatile compounds from LOX pathways

Attribute	Compound
Bitter	Secoiridoid compounds, 1-penten-3-one
Pungent	Secoiridoid compounds, 1-penten-3-one
Leaf	Secoiridoid compounds, 1-penten-3-one
Fruity (green olives)	*cis*-2-penten-1-ol
Lawn	*Trans*-2-hexenal
Walnut husk	*cis*-3-hexenyl acetate, *trans*-2-pentenal, secoiridoid compounds
Tomato	Hexan-1-ol, 1-penten-3-one
Almond	*cis*-2-penten-1-ol, hexanal

longer times because of the reduction of the concentrations of secoiridoid compounds and 1-penten-3-one. Lower intensities of these attributes should be found at 35°C, because temperature more than time of malaxation influences the amounts of the mentioned compounds.

Fruity sensation should not show important effects because of poor changes in concentration of *cis*-2-penten-1-ol. The lawn perception should change depending on the amount of *trans*-2-hexenal, that was the compound showing the better correlation. Tomato perception, because of the more considerable decrease of 1-penten-3-one at 35°C should be negatively affected by temperature more than malaxation time.

The drastic reduction of secoiridoid compounds and *cis*-hexenyl acetate should be responsible for the weakening of the intensity of walnut husk perception, in particular in oils obtained at 35°C. On the other hand, it was expected that almond sensation, dependent on *cis*-2-penten-1-ol and hexanal contents, was perceived with a stronger intensity with the prolonging of paste malaxation; temperature should not have noticeable effects because of its poor influence on the production of these compounds.

The evaluation of sensory attributes was performed by eight tasters fully trained in the evaluation of green attributes, of oils extracted from fruits of Frantoio and Coratina cvs by using different operative conditions of paste malaxation. Fig. 2 shows the intensities of attributes perceived by tasters for oils from Frantoio cv at two different temperatures and at the malaxation times more frequently used in the oil extraction process.

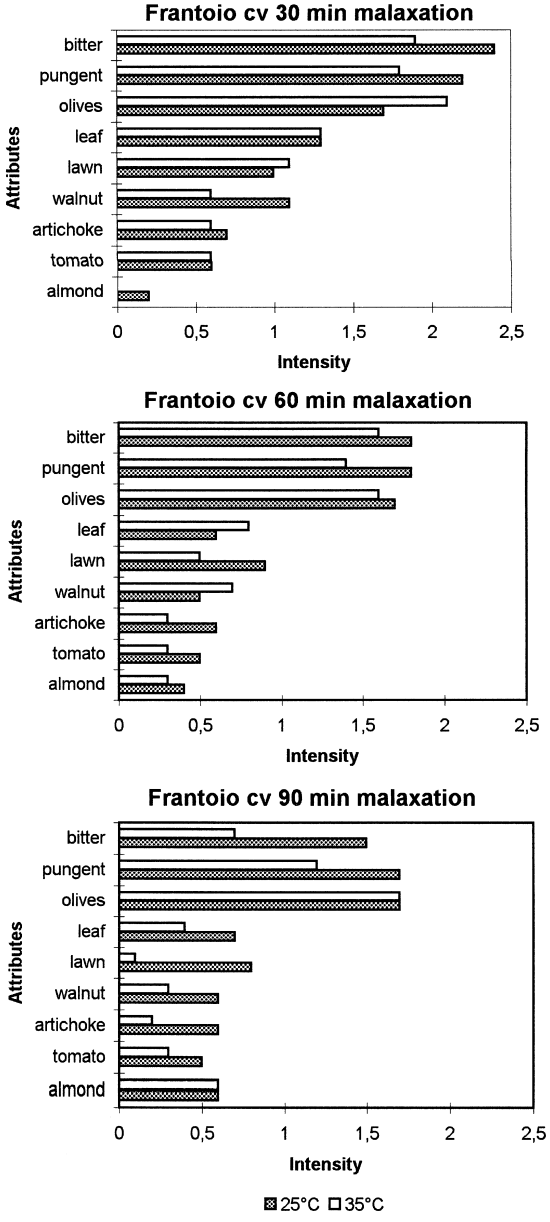

Fig. 2. Intensities of attributes perceived by assesors for oils from Frantoio cv extracted at 25° and 35°C and at 30, 60 and 90 min, respectively.

Table 4
Standard deviation, mean, minimum and maximum values (as ppm of nonan-1-ol) of the amounts of metabolites from conversion of some amino acids detected in 200 oil samples of high quality

Compound	Mean	S.D.	Min	Max
3-Methyl butanal	2.5	2.58	0.1	13.4
2-Methyl-butanal	2.0	1.78	0.1	8.3
2- + 3-Methyl-butan-1-ol	6.5	5.06	0.1	22.0
2-Methyl propan-1-ol	2.4	2.41	0.0	9.9
Propanoic acid	0.0	0.00	0.0	0.0

Results obtained fitted well with all expectations. Tasters generally perceived a weakening of flavour with both the prolonging of malaxation and the raising of malaxation temperature from 25 to 35°C. The temperature was mainly responsible for the flattening of green attributes such as lawn, leaf and tomato, and also for bitter and pungent perceptions. A similar trend was observed for Coratina cv.

In previous research (Angerosa et al., 2000) no relationship was found between artichoke sensation and secoiridoid and volatile compounds from LOX pathways; nevertheless assessors perceived a weakening of this attribute.

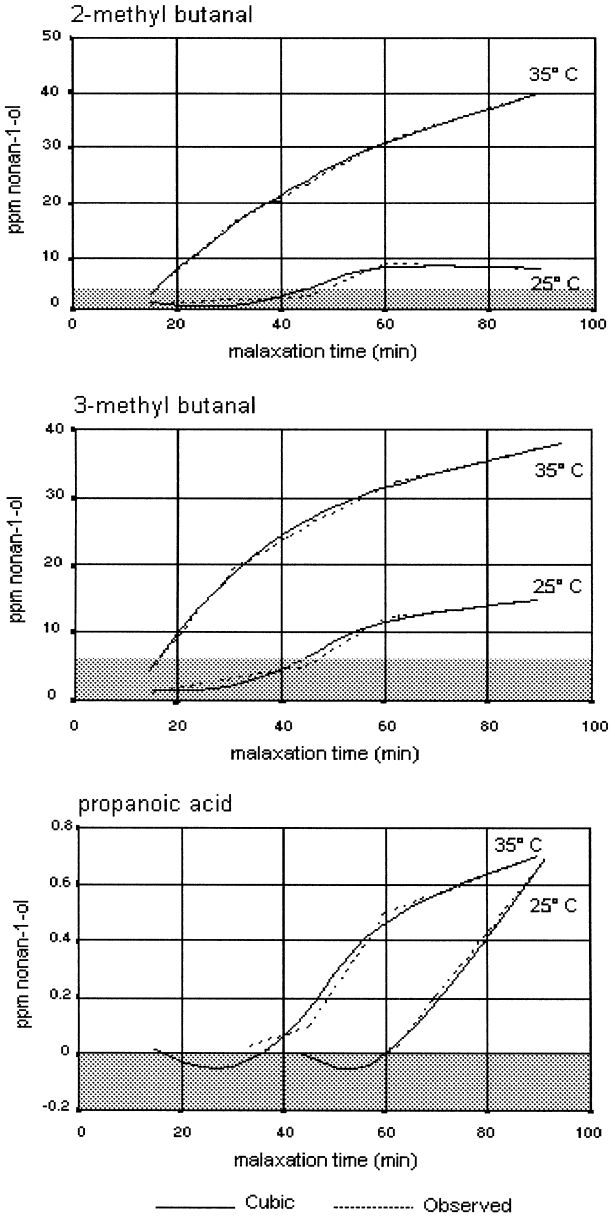

Fig. 3. Graphical plots of detected (dotted line) and extimated curves (continuous line), of concentrations of the metabolites from amino acid conversion for oils of Frantoio cv with respect to the operative conditions of malaxation. A light grey area for each metabolite delimits the lower and the upper limit amounts that include 90% of 200 high quality samples.

Olive oil quality depends on the presence of other metabolites and especially on their concentration. Intermediate forms of the pathway of conversion of valine, leucine and *iso*-leucine (2-methyl and 3-methyl butanal, their corresponding alcohols, 2 methyl butanoic acid, 3-methyl butanoic acid and propanoic acid) were identified in some virgin olive oil aromas. The first metabolites of this pathway are 2-methyl and 3-methyl butanal, enzymatically reduced to their corresponding alcohols. Quite large amounts of 2-methyl butanoic acid and 3-methyl butanoic acid and of propanoic acid are usually detected only in oils obtained from fruits damaged and/or stocked for long periods (Angerosa et al., 1996).

Generally, in oils obtained from fresh healthy olives, the concentrations of branched aldehydes and alcohols are low. The quantitative determination of compounds arising from conversion of amino acids was carried out on 200 high quality olive oil samples obtained regardless of cultivar and oil extraction system adopted (from various cultivars with different oil extraction systems). Table 4 lists, as ppm of nonan-1-ol (internal standard), the mean value, standard deviation, minimum and maximum values detected for the mentioned metabolites of the 200 samples examined. Data confirm the absence of acids in head-space composition and the low concentration of 2-methyl butanal and 3-methyl butanal. Branched alcohols showed amounts twice those of corresponding aldehydes.

In addition, the application of statistical procedures indicated that 90% of oil samples fell between 0.1 and 12.9 for 2-methyl butan-1-ol + 3-methyl butan-1-ol, 0.1 and 4.5 for 2-methyl butanal, 0.1 and 5.9 for 3-methyl butanal, 0.0 and 6.5 for 2-methyl propan-1-ol. Therefore, the lower and upper values of the amount of each metabolite identified the range of concentration that included olive oils to be certainly considered of high quality.

Table 5 shows the amounts, expressed as ppm of nonan-1-ol, of metabolites from conversion of valine, leucine and isoleucine detected in oils obtained with different conditions of paste malaxation.

A close inspection of data in Table 5 showed that 2-methyl and 3-methyl butanal, their corresponding alcohols and 2-methyl butan-1-ol accumulated during the malaxation. The greatest increases were observed for branched aldehydes, whereas the concentrations of the remaining compounds poorly changed. The accumulation of 2-methyl butanal and 3-methyl butanal is much greater when the olive paste is malaxed at 35°C, whereas a low increase is detected for corresponding alcohols . It is noteworthy that propanoic acid was absent until 60 min of malaxation in oils from both cultivars at 25°C and appeared between 30 and 45 min at 35°C, giving

Table 5
Amounts, expressed as ppm of nonan-1-ol, of metabolites from conversion of valine, leucine and isoleucine in oils obtained at the different times and temperatures adopted during paste malaxation

	CORATINA *cv*									
	25°C					35°C				
ppm nonan-1-ol	15′	30′	45′	60′	90′	15′	30′	45′	60′	90′
2-methyl butanal	1.0	2.1	6.1	9.9	8.6	3.3	14.0		18.6	30.8
3-methyl butanal	1.3	4.1	8.7	14.7	14.8	5.1	20.4		25.3	38.6
2-methyl 1-propanol	1.2	0.9	0.8	0.9	0.8	0.9	1.0		1.2	1.4
2- + 3-methyl butan 1-ol	5.9	6.4	6.4	7.4	7.1	7.9	9.4		12.4	14.6
propanoic acid	0.0	0.0	0.0	0.0	0.4	0.0	0.1		0.4	0.7
	FRANTOIO *cv*									
	25°C					35°C				
ppm nonan-1-ol	15′	30′	45′	60′	90′	15′	30′	45′	60′	90′
2-methyl butanal	0.8	1.8	2.3	8.5	7.4	3.3	15.7	23.5	31.2	40.1
3-methyl butanal	1.1	3.1	5.0	12.1	14.9	4.3	19.2	26.3	32.1	37.6
2-methyl 1-propanol	0.3	0.4	0.5	0.5	0.6	0.5	0.9	0.8	1.0	1.2
2- + 3-methyl butan 1-ol	7.6	6.7	7.7	10.4	10.4	6.5	7.6	10.1	9.9	14.3
propanoic acid	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.1	0.5	0.7

evidence of a marked deterioration of olive paste. The other acids were never produced in any experiments performed.

Table 5 shows that the trend of increases of 2-methyl butanal and 3-methyl butanal was the same for both cultivars and that their concentrations at 90 min were similar. The levels of 2-methyl butanal and 3-methyl butanal, considered as upper limits (4.5 and 5.9 as ppm of nonan-1-ol, respectively) for oils in which high quality was assured, were overstepped at 45 min for Coratina and 60 min for Frantoio cv when paste temperature was kept at 25°C, whereas the amounts of branched aldehydes at 30 min for both cultivars fell outside upper limits when the temperature was 35°C. In addition, propanoic acid, always absent in all 200 samples of high quality oils, started to be produced at temperature of 35°C after 30 min (Coratina) and 60 min (Frantoio) of malaxation and only after 90 min when temperature was low (25 °C).

Furthermore, in all experiments performed, the extreme malaxation conditions never caused the production of high levels of 2-methyl butan-1-ol + 3-methyl butan-1-ol and 2-methyl propan-1-ol. Noticeable amounts of 2-methyl butan-1-ol + 3-methyl butan-1-ol were related to "fusty" defects by results of previous researches (Angerosa et al., 1996), but in this case, oils also showed higher concentrations of corresponding aldehydes and of 2-methyl propan-1-ol than oils without sensory defects. Therefore, the concentrations of only branched aldehydes could be regarded as a mark of unsuitable conditions of time and/or temperature during the malaxation.

Because of the above cosiderations in relation to technological conditions adopted during the malaxation, regression curves were constructed using a SPSS statistical package for 2-methyl and 3-methyl butanal and propanoic acid for each cv at 25 and 35°C temperatures. Malaxation times were assumed as independent variables, whereas the amount obtained for the two compounds represented the dependent variables.

The output from the procedure applied to find the best curve fit showed that the cubic equation better described the increase of 2-methyl and 3-methyl butanal during the malaxation at both adopted temperatures and of propanoic acid at 25°C, whereas a linear regression curve better fitted the propanoic acid increase at 35°C. Moreover, the software provided the graphical plot of both values detected (dotted line) and estimate curve (continuous line), giving an immediate representation of changes of each metabolite with respect to operative conditions of malaxation. In Fig. 3, plots of concentrations of metabolites from amino acid conversion were drafted for oils from Frantoio cv. A light grey area delimited the lower and the upper limit amounts for each metabolite that included 90% of 200 high quality oil samples. Consequently, levels of 2-methyl butanal, 3-methyl butanal and propanoic acid falling outside these grey areas were not associated with oils of high quality.

The values considered as upper limit for 2-methyl butanal and 3-methyl butanal, respectively, were always overstepped when extreme malaxation conditions (too long time and especially temperature about 35°C) were adopted in oil extraction so that they could not be con-

sidered advisable for obtaining oils of high quality. Low temperature (about 25°C) allowed olive pastes to be malaxed for 30–45 min depending on their rheological characteristics, whereas temperatures of about 35°C forced the shortening of malaxation time to 10–15 min to obtain oils without sensory defects.

The quantitative evaluation of compounds from amino acid conversion was in complete agreement with the conclusions drawn from sensory data and from analytical determination of volatile composition and secoiridoid compounds.

4. Conclusions

Evidently malaxation time mainly promoted the increase of C_6 and C_5 carbonyl compounds and alcohols, and negatively affected the intensity of some sensory attributes and the concentration of secoiridoid compounds.

Temperature was mainly responsible for: (i) the sensory flattening of oils, (ii) very considerable losses of secoiridoid compounds, (iii) the marked decrease of concentration of C_6 esters, very important contributors of delicate green perceptions, and of *cis*-3-hexen-1-ol which gives pleasant real green sensations, (iv) the increase of hexan-1-ol and *trans*-2-hexen-1-ol considered elicitors of less attractive perceptions (Aparicio et al., 1994; Bedukian, 1971; Aparicio, Morales & Alonso, 1996); (v) the production of very high contents of 2-methyl butanal and 3-methyl butanal through the activation of the amino acid conversion pathway.

Low temperatures are always recommended for the malaxation step, whereas the choice of the optimal malaxation time should be made so that a satisfactory yield and a good quality of oil can be achieved. Times ranging between 30 and 45 min, according to rheology of the olive pastes, seem to satisfy these requirements, because compounds responsible for attractive perceptions, such as esters, are still present at high level, and concentrations of those giving unpleasant sensations such as *trans*-2-hexen-1-ol and hexan-1-ol are rather low. In addition, the amount of secoiridoid compounds is great enough to assure a suitable shelf-life of the product and the content of branched aldehydes is in the range typical of olive oils of high quality.

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