Characterization of Olive Ripeness by Green Aroma Compounds of Virgin Olive Oil

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The evolution of the volatile compounds responsible for virgin olive oil green aroma has been studied in samples obtained from four olive fruit varieties (Arbequina and Picual, Spain; Koroneiki, Greece; and Coratina, Italy), picked at three different stages of ripeness during two consecutive crops. The volatile compounds hexanal, (*E*)-3-hexenal, (*Z*)-3-hexenal, (*E*)-2-hexenal, hexyl acetate, (*Z*)-3-hexenyl acetate, hexan-1-ol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol were considered in this study. The sensory significance of these volatile compounds has been stated by their odor activity value (OAV), while their synergic relationships with green sensory attributes have been analyzed by principal component analysis. The stages of ripeness of each variety, with the whole set of volatile compounds, were characterized by cluster analysis. Detailed information corresponding to each volatile compound for each stage of ripeness, after fuzzy filtering of the quantitative data, has also been established. Alcohols produced from linolenic acid (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol, and hexanal and hexyl acetate—both produced from linoleic acid—are major contributors to the ripeness characterization. The results were verified by applying multiple regression to the quantitative values of volatile compounds of a test set of eight Arbequina samples picked each fortnight from unripened to over-ripened olives. From the results of this study it can be concluded that the unripe stage is the best characterized, although the result in prediction for three stages of ripeness was an adjusted R^2 value of 0.98.

Keywords: *Olea europea L.; virgin olive oil; volatiles; green sensory note; ripeness; statistics; fuzzy logic*

INTRODUCTION

Virgin olive oil is the oil extracted from the fruit of the olive tree, *Olea europea* L. This vegetable oil is consumed without a further refining process, so retaining volatiles and other minor compounds that give rise to a fragrant and delicate flavor that has been appreciated by consumers since ancient times.

In previous works (Aparicio et al., 1994; Aparicio and Morales, 1995) different sensory notes responsible for virgin olive oil flavor were established. The most remarkable sensory perceptions for consumers are green (leaves, grass), sweet, fruity, ripe fruit, ripe olives, undesirable, and bitter-pungent (IOOC, 1987). Most of these sensory perceptions are produced by volatile compounds (Flath et al., 1973; Morales et al., 1995; Aparicio et al., 1996b) that stimulate the olfactory receptor cells of the nose, giving rise to a wide panoply of sensory notes (Maruniak, 1988). From all of them, the green sensory perception is of great importance because the fragrant flavor of the virgin olive oil is produced by the balance between green and fruity notes (Morales et al., 1996). It has been stated that aliphatic C_6 compounds and the corresponding hexyl acetates (Guth and Grosch, 1991, 1993; Morales et al., 1996) are the main contributors to the "unripe" component of the fruit flavor. Moreover, almost all volatile compounds responsible for green sensory notes are the major components of the virgin olive oil headspace (Morales et al., 1994).

These compounds are biosynthesized from C_{18}-unsaturated fatty acids in plants following the lipoxygenase (LOX) pathway (Vick and Zimmerman, 1987; Hatanaka et al., 1987). This pathway involves the actuation of different enzymes giving rise to different amounts of aldehydes, alcohols, and hexyl acetates (AIR, 1994), all of which have sensory properties and so contribute to the overall flavor. In the case of olive fruits, it has been demonstrated that the LOX pathway promotes the formation of C_6 volatile compounds against C_9 volatile compounds and, in consequence, a great amount of volatile compounds responsible for green sensory notes (Morales et al., 1994) can be found in fresh and high-quality virgin olive oils.

The first aim of this work was to determine whether there were differences in the "green" volatile content of virgin olive oil samples collected from different crops and also the evolution of each volatile compound. The second was to characterize each stage of ripeness by their green volatile content using mathematical procedures. The relationships between the green volatile compounds and the sensory attributes evaluated by several sensory panels were also studied. Eventually, a test set was used to check the relationship between selected green volatile compounds and the stages of ripeness.

MATERIALS AND METHODS

Samples. During two consecutive crops, virgin olive oil was properly obtained from fresh, healthy fruits of good quality collected at three stages of ripeness: at the beginning of the

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harvest period when fruits are of green color (unripe), in the middle of the harvesting (medium ripe), and at the end when fruits are of black color (over-ripe) (FLAIR, 1991). As olive fruits are drupes that change color during ripening, depending on the variety, four varieties were selected—Arbequina and Picual (Spain), Coratina (Italy), and Koroneiki (Greece). They were chosen to represent a substantial proportion of the bottled virgin olive oil trade (Morettini, 1950; Humanes, 1993) and being completely different with regard to sensory evaluation (Aparicio et al., 1994) or chemical composition (Morales and Aparicio, 1993a). Three samples of each variety per crop were studied corresponding to unripe, medium ripe, and over-ripe stages of ripeness, totaling 24 samples. These stages of ripeness were detected by applying the procedure suggested by EOC (1976)—a linear algorithm based on the color of the skin and pulp of olives. In general, the lowest value (0) corresponds to intense green skin, while the highest corresponds to intense black color of the pulp (7). Some standardized changes qualifying the stages of ripeness were applied to varieties Arbequina and Coratina as they did not reach the intense black color. Unripe olives were characterized by values 0−1, medium ripe by 4−5 (maximum yield), and over-ripe to 7 (Frias et al., 1991).

Another set of eight virgin olive oil samples of Arbequina variety was used as a test set to determine the feasibility of the quantitative measure of certain volatile compounds to indicate the maturity of olive fruits from which oils are obtained. The samples were picked up each 10−15 days from October to January, the first being the most unripe (value 0) and the last the most over-ripe (value 7), totaling eight different stages of ripeness.

Olive oil samples were produced by selected commercial (Italy and Greece) and research (Spain) plants; >500 kg was used in each experiment. The production of oils was standardized in relation to extraction technology, although some slight differences were detected in the time of malaxing, temperature of water added, and temperature of decantation. The transportation of the olives from the field to the plant was carried out in jute sacks for Greece and in containers for Italy and Spain. The plants were cleaned before and after each experience to avoid contamination from other olives. The olives were transported to the plants, and olive oil was elaborated, on the same day in Spain and within 24 h in Italy and Greece. The samples of olive oil were shipped under strict conditions and always by courier. All virgin olive oil samples were freeze-stored until the moment of analysis.

Factorial analysis was previously used to determine the analytical errors and choice the optimum scheme of the instrumental method (Morales and Aparicio, 1993b). The analysis of variance allowed calculation of the method error and, so, a decision as to which is the optimum number of analysis. The cross-point between the cost (laboratory expenses and time) and variance curves was used in deciding the optimum number of replicates, to avoid subjective decisions. The cross-point between both curves corresponded to two analyses with a mean coefficient of variation of 0.15 for the whole chromatogram (65 chemical compounds). All of the chemical compounds described in the paper have a coefficient of variation of <0.1 except (*E*)-3-hexen-1-ol, for which the coefficient of variation is 0.18. All samples were analyzed in duplicate.

Dynamic Headspace Gas Chromatography. Volatile compounds of virgin olive oil samples were analyzed by a dynamic headspace technique previously reported (Morales et al., 1994; Aparicio and Morales, 1994). Samples of 0.5 g were heated at 40 °C and swept with N_2 (200 mL/min) for 15 min, and the volatiles were adsorbed onto a Tenax TA trap (Chrompack, Middleburg, The Netherlands) at room temperature. A Chrompack thermal desorption cold trap injector (TCT) was employed to carry out the thermal desorption of the trapped volatiles by heating at 220 °C for 5 min. The volatiles were then condensed onto a fused silica trap cooled at −110 °C with liquid nitrogen for 5 min just before injection, which was carried out by flash heating of the cold trap at 170 °C, at which it was held for 5 min. The volatiles were transferred onto a fused silica Supelcowax 10 column (60 m × 0.32 mm i.d., 0.5 μm film thickness) (Supelco, Bellefonte, PA). The oven temperature was held at 40 °C for 4 min and programmed to rise at 4 °C/min to a final temperature of 240 °C, at which it was held for 10 min. A Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA) with an FID detector was employed. Quantification was carried out using isobutyl acetate as internal standard.

Gas Chromatography/Mass Spectrometry. Volatiles were obtained using the adsorption−desorption technique described above. Twenty-five grams of virgin olive oil and 30 min of adsorption time were used to achieve a more concentrated sample. A J&W DB-WAX fused silica column (60 m × 0.25 mm i.d., 0.25 μm film thickness) was employed (J&W Scientific, Folsom, CA). An MS 30/70 VG mass spectrometer (VG Analytical, Manchester, U.K.) and a VG 11/250 data system were used for mass spectrometric analyses. Operating conditions were as follows: temperature, programmed 15 min at 40 °C, then from 40 to 220 °C at 1 °C/min; carrier gas flow rate, helium at 1 mL/min. The end of the fused silica column was inserted directly into the ion source block. The spectra were recorded at an ionization voltage of 70 eV and at an ion source temperature of 200 °C.

Sample components were verified by comparison of mass spectral data with those of authentic reference compounds. (*E*)-3-Hexenal, for which a standard was not available, was tentatively identified by mass spectrum matching using the NBS mass library collection. In the case of (Z)-3-hexenal, this compound was synthesized (Hatanaka et al., 1992) and its mass spectrum obtained.

Sensory Properties of Volatile Compounds. *HRGC Eluate Sniffing.* To assess the aroma notes corresponding to olive oil volatile compounds, a high-resolution gas chromatography (HRGC) sniffing technique was applied to virgin olive oil samples (Morales et al., 1994). The effluent of the GC column was split 1 to 10 to the detector and the sniffing port, respectively. The odor-active regions of the eluate were evaluated, and their aroma notes were assigned by five assessors— two with >10 years of experience and three who were habitual consumers of virgin olive oil. The odor descriptions were noted on a form with a preprinted time scale; assessors did not see the chromatogram. Assessors basically agreed on the odors of volatiles, although different semantic terms were used to describe some of them. A consensus-building discussion was held with assessors to decide the final sensory descriptors.

Threshold Values and Tasting of Pure Compounds. Odor threshold values of volatile compounds in a matrix of deodorized sunflower oil were found in the bibliography. In the case of hexyl acetate, hexan-1-ol, (*E*)-2-hexen-1-ol, and (*E*)-3-hexen-1-ol, the odor thresholds were determined by the authors using the method described by Guth and Grosch (1993).

The same assessors also carried out the smelling and tasting, in duplicate, at room temperature of four pure volatile compounds to assess their sensory properties: hexan-1-ol (Merck, Darmstadt, Germany), (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*E*)-3-hexen-1-ol (Aldrich, Milwaukee, WI). The volatile compounds were previously diluted in water or paraffin oil, depending on their solubility, to the same approximate concentration as found in virgin olive oil samples. The tasting of these volatiles allowed their characterization as astringent and bitter [(*E*)-2-hexen-1-ol and (*E*)-3-hexen-1-ol], rough (hexan-1-ol), and sharp, bitter, astringent [(*E*)-2-hexenal].

Sensory Analysis. Three sensory panels working in an EC FLAIR Project (FLAIR, 1991) carried out the quantitative descriptive analysis (Stone et al., 1974) of the virgin olive oil samples. The British panel used a sensory profiling (Lyon and Watson, 1994), while the Italian and Greek panels strictly followed the EC regulation (EC, 1991) to evaluate the samples. Table 1 summarizes the most relevant characteristics of each panel.

From the total number of sensory attributes evaluated by the panels, five green attributes were selected for this work: green fruity, green-bitter, banana skins, olive fruity (ripe and green), and green. The means of the triplicate evaluations of

Table 1. Basic Characteristics of Panels

nationality	Greek	British	Italian
no. of assessors	14	9	10
consumer	H[a]	P[a]	H
total attributes	15	26	16
scale	S[b]	U[b]	S
scores	1−5	100 mm	1−5

[a] H/P, habitual/potential virgin olive oil consumers. [b] S/U, structured/unstructured.

each attribute made by the assessors were calculated and then the mean of assessors for each attribute. Thus, there was a value of each attribute for every sample. Data were standardized by *Z*-score since there were differences between them because the assessors used different scales and scores (Table 1).

Statistical Analysis. All variables had an almost normal distribution, so that no transformation had to be applied to the data. After normalization by *Z*-score (mean 0, standard deviation 1), all mathematical procedures were applied.

Stages of ripeness were characterized using fuzzy logic procedures previously reported (Calvente and Aparicio, 1995; Aparicio et al., 1996a). Quantitative data corresponding to the three stages of ripeness were filtered by this fuzzy logic procedure to separate the information corresponding to each stage without interference from the others.

The differences between the stages of ripeness for each variety were pointed out using cluster analysis (CA) [city-block (Manhattan) distance and Ward's method algorithms] (Massart and Kaufman, 1983). The unsupervised procedure of cluster analysis was used to have a preliminary graphical information of the possibilities of distinguishing the stages of ripeness using the whole set of volatiles simultaneously.

The mean scores of the green attributes sensory evaluation and of the quantitative data for each compound were subjected to principal component analysis (PCA) (Tabachnick and Fidell, 1983). Cross-validation repeated three times with different cancellation matrices always detected three significant components, explaining 71% of the total variance. PCA has also been used to detect differences between the stages of ripeness.

Multiple linear regression analysis (MLRA) was applied on the test set (Tabachnick and Fidell, 1983). The MLRA procedure yielded information about those volatile compounds explaining the time of harvesting. Volatiles were selected by taking into account the information of fuzzy filtering. Volatiles were selected by taking into account the values of *F*-to-enter of *F*-distribution at $p = 0.05$.

All mathematical procedures, except fuzzy logic, were carried out using the STATISTICA package (Statsoft, 1995) on a Pentium computer. The fuzzy filter was written in Fortran (Calvente and Aparicio, 1995). In this procedure all integrals were calculated by using Gauss−Legendre quadrature (Press et al., 1992), with a variable number of points depending upon the interval width. To have a version with higher speed of execution, the fuzzy filter procedure was implemented in an Alpha DEC-400 workstation.

RESULTS AND DISCUSSION

Ten volatile compounds were considered in this study. They were C_6 aliphatic compounds corresponding to aldehydes, alcohols, and acetates: hexanal, (*E*)-3-hexenal, (*Z*)-3-hexenal, (*E*)-2-hexenal, hexyl acetate, (*Z*)-3-hexenyl acetate, hexan-1-ol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol. Table 2 shows corresponding odor descriptions obtained by sniffing the samples; these compounds gave a green-type description covering a wide range from mild green to intense cut grass in accordance with the results obtained using pure compounds (Hatanaka et al., 1992). Odor thresholds of the volatile compounds and their OAVs in each different stage of ripeness are also given.

Figure 1 shows the mean values (micrograms per kilogram) of the volatile compounds at three stages of ripeness for all varieties. We have calculated the mean of varieties as the objective is not to analyze the evolution of each variety independently but to describe the relationship between the lipoxygenase cascade and the maturity regardless of the variety analyzed.

A decrease in the values was observed in almost all cases from unripe to over-ripe. In the case of hexyl acetate an increase was obtained at the medium stage of ripeness, and in the case of hexan-1-ol a decrease was produced at this stage, although the difference between the medium and over-ripe stages is <15%. Hexan-1-ol is the precursor of hexyl acetate through the LOX pathway, since the former is the substrate of the alcohol acetyl transferase that is the enzyme responsible for the formation of the ester. The decrease of hexan-1-ol and simultaneous increase of hexyl acetate could be related with a greater activity of the enzyme responsible for the ester formation in the medium stage of ripeness.

The production of (*E*)-3-hexenal follows an opposite behavior, the highest values being at over-ripe stage and the lowest at unripe. It is also noteworthy that the content of (*E*)-2-hexenal is by far the highest in all varieties and (*E*)-3-hexen-1-ol presents a very low concentration for all samples.

A detailed analysis of varieties agrees basically with the information given in Figure 1. However, we have detected a certain influence of climate in the production of volatiles during the stages of ripeness. Coratina variety showed a linear decrease in the content of (*E*)-2-hexenal, during its ripening, in the samples of the first year, while a small increase was detected at medium ripeness (17% over the values of unripen olives) in samples of the second year. These results agree with the results from Montedoro et al. (1978) for other Italian varieties (Bonino, Moraiolo, and Frantoio), and it could be explained by the dry climate of that year, which also agrees with the results of AIR (1994) for irrigated and nonirrigated orchards. In fact, Figure 1 shows, for the whole set of varieties, less decrease between unripe and medium ripe oils than between medium ripe and over-ripe oils.

All of these volatiles have a green odor description (Table 2) (Hatanaka et al., 1992), but the sensory wheel (Aparicio et al., 1996b) stated that some of them could also contribute to other sensory perceptions related to taste. (*E*)-2-Hexenal and (*E*)-3-hexen-1-ol were related to bitter attributes, while hexan-1-ol and (*E*)-2-hexen-1-ol were related to undesirable attributes. The results of tasting pure compounds confirmed that these compounds contribute to both perceptions, aroma and taste, of virgin olive oil.

These volatile compounds are produced through the LOX pathway. In this pathway different enzymes are involved, giving rise to different volatile compounds. The enzymatic activity of these enzymes is closely related to the variety, as can be concluded from the total green volatile content found in different varietal virgin olive oils (Morales et al., 1996).

However, each variety has a different behavior in terms of ripeness. In the case of Picual, which ripens very quickly, and Koroneiki, with an early ripeness, the limits of the medium ripe degree of ripeness is not quite clear (Figure 2a) despite the method used to establish the degree of ripeness being objective enough (EOC, 1976). Arbequina, which is characterized by a minimal

Table 2. Mean Retention Times, Descriptions, Odor Thresholds, and Odor Activity Value Ranges of the C_6 Aliphatic Compounds and Hexyl Acetates

code	volatile compound	t_R (min)	odor descriptors	odor threshold (μg/kg)	OAV		
					UR[a]	MR[a]	OR[a]
1	hexanal	16.5	green, apple, green fruit	75[b]	3.9	2.5	1.4
2	(*E*)-3-hexenal	19.5	artichoke, green, flowers	450[b]	1.0	1.2	1.5
3	(*Z*)-3-hexenal	19.9	green, cut grass	2.8[b]	155	140	97
4	(*E*)-2-hexenal	23.1	green, fruity, almonds	1125[b]	12.6	11.1	5.6
5	hexyl acetate	24.8	fruity, sweet	1040[c]	0.1	0.2	0.2
6	(*Z*)-3-hexenyl acetate	26.2	fruity, green leaves	750[b]	0.1	0.1	0.1
7	hexan-1-ol	27.7	fruit, banana, soft	400[c]	1.2	1.1	1.2
8	(*E*)-3-hexen-1-ol	28.2	green	1500[c]	0.02	0.02	0.01
9	(*Z*)-3-hexen-1-ol	29.1	grass, banana	6000[b]	0.1	0.1	0.1
10	(*E*)-2-hexen-1-ol	29.8	green, grassy, sweet	8000[c]	0.1	0.06	0.06

[a] UR, unripe olives; MR, medium ripe; OR, over-ripe. [b] Odor thresholds in freshly refined sunflower oil (Guth and Grosch, 1993; Grosch, 1994). [c] Odor thresholds in sunflower oil determined for this work.

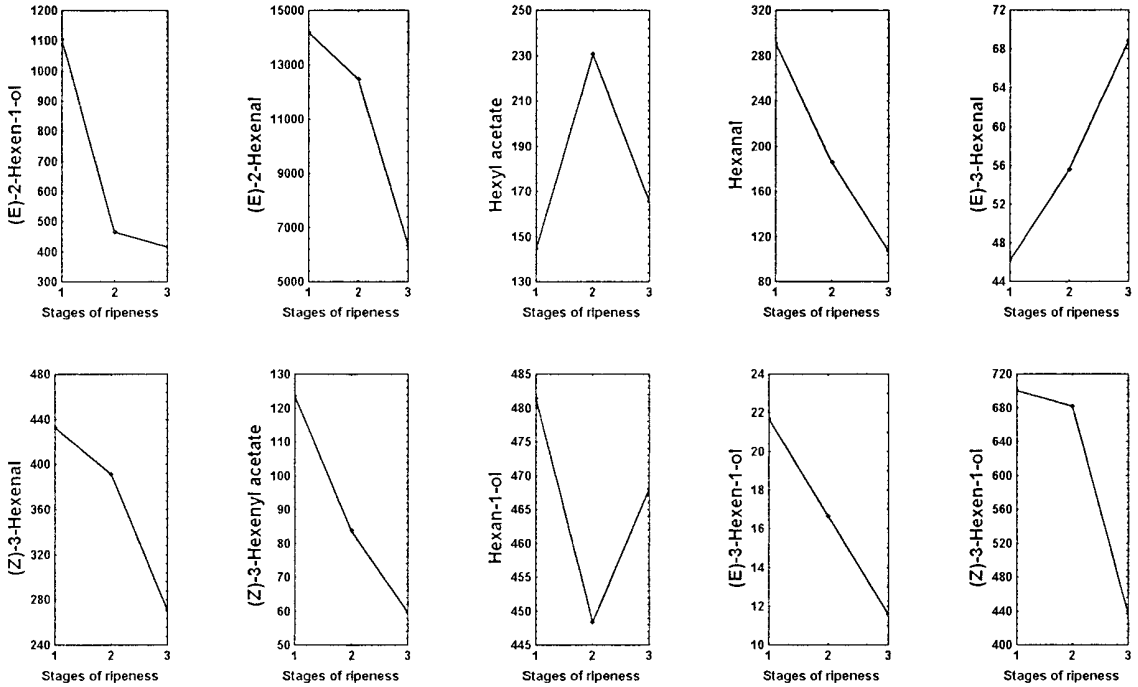

Figure 1. Changes of the concentration (micrograms per kilogram) of volatile compounds with ripeness. Stages of ripeness: l, unripe; 2, medium ripe; 3, over-ripe. Mean values of the four varieties studied are plotted.

change of color, showed a linear evolution of its ripeness, making it easy to distinguish its ripeness (Figure 2b). Coratina, which ripens late, showed greater differences of its volatile compounds at the overripe stage versus the other two stages (Figure 2c). In fact, the amount of (*E*)-2-hexenal for this olive oil variety decreases abruptly when olives are picked over-ripe.

Once it was established that the green volatiles, except (*E*)-3-hexenal, decrease with ripeness in all of the varieties studied, the next step was to study the information of each compound corresponding to each stage of ripeness. However, olive oil chemical composition is not an invariable parameter. Olive variety has the greatest influence up to the point that varieties can be characterized by either chemical compounds (Solinas et al., 1988; Morales and Aparicio, 1993a,b; Aparicio and Alonso, 1994; Aparicio et al., 1997) or sensory attributes (Aparicio et al., 1996a).

A study of ripeness, in which different olive varieties are involved, should take into account the fuzziness of the ripeness evaluation of the olives and, in consequence, it seems reasonable that the characterization of the stages of ripeness derives from fuzzy logic rather than classical arithmetic. This mathematical procedure has demonstrated its usefulness in determining not only the authentication of foodstuffs (Calvente and Aparicio, 1995) but also the sensory attributes (Aparicio et al., 1996a) or chemical compounds (Aparicio et al., 1997) qualifying the European virgin olive oils.

A previously developed fuzzy filter algorithm (Calvente and Aparicio, 1995), based on an *S*-number (Kandel, 1986), was applied to the initial distributions of the quantitative values of each compound, generated from its minimum, mean, and maximum values at each stage of ripeness. The filter removes the overlapped quantitative values (or interferences) of each stage of ripeness and assigns a different zone of the distribution to each stage of ripeness without contribution of the others. Thus, the possibility values can be used in an absolute sense to get conclusions—higher values mean not only a strong belonging to one stage of ripeness but also a weak belonging to the others.

Figure 3A shows the distribution function for the set of 10 volatile compounds. Minimum, mean, and maximum quantitative values of each compound at each stage of ripeness were used to generate each distribu-

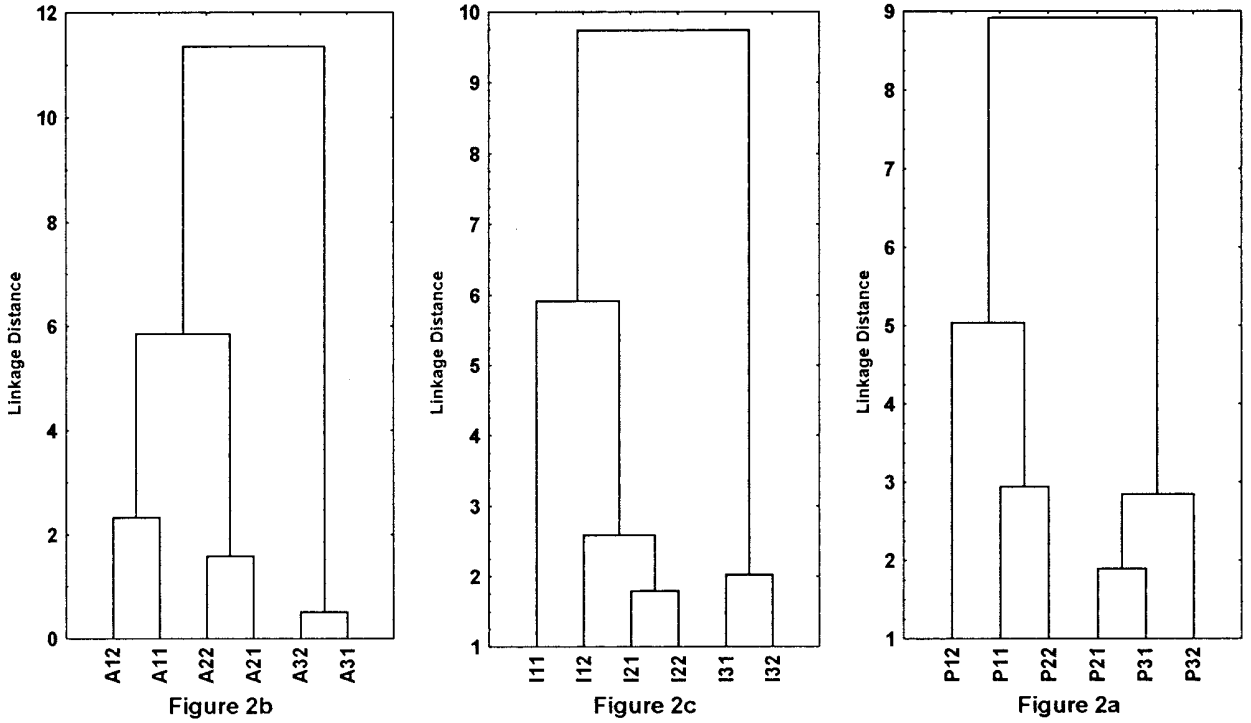

Figure 2. Results of cluster analysis showing the characterization of stages of ripeness of varieties (using all volatiles described): (a) Picual; (b) Arbequina; (c) Coratina; P11, Picual unripe first-year crop; P12, Picual unripe second-year crop; P21, Picual medium ripe first-year crop; P22, Picual medium ripe second-year crop; P31, Picual over-ripe first-year crop; P32, Picual over-ripe second-year crop. The same procedure has been followed to codify Arbequina (A) and Coratina (I).

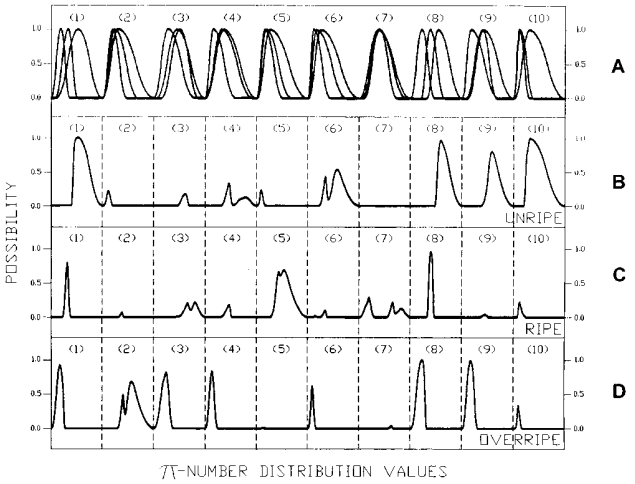

Figure 3. Distributions of 10 volatile compounds values: (A) nonfiltered values; (B) unripe filtered values; (C) ripe filtered values; (D) over-ripe filtered values. Codes in parentheses correspond to volatiles described in Table 2.

tion. The fuzzy filter made it possible to isolate the pure contributions of each volatile compound for unripe, medium ripe, and over-ripe maturities (parts B, C, and D of Figure 3, respectively).

Additional information can be obtained from the shape of the resulting filtered function. Thus, it may be stated that height symbolizes the selectivity, since whenever a band becomes higher in some range, the contribution of the others in it is lesser. The width of the functions could be related to sensitivity since, as width increases, the ability to characterize a given stage of ripeness becomes greater.

Bearing in mind the above considerations, each compound can be differentiated according to its selectivity and sensitivity levels. The volatile compounds hexanal (**1**), (*E*)-3-hexen-1-ol (**8**), (*Z*)-3-hexen-1-ol (**9**), and (*E*)-2-hexen-1-ol (**10**) show (i) a great, simultaneous sensitivity and selectivity for the unripe stage, (ii) only a high selectivity for the over-ripe stage, and (iii) a globally poor response in the case of the medium ripe stage. Therefore, they can be used to properly characterize the first stage of ripeness.

The medium stage of ripeness could not be characterized using these compounds—only hexyl acetate (**5**) shows an adequate level of selectivity and sensitivity. This conclusion is also logical since we are studying different varieties with different evolutions of ripeness (Figure 2), so the volatile content for this stage could be influenced by the other stages.

The over-ripe stage can be characterized with low values of hexanal (**1**), (*Z*)-3-hexenal (**3**), (*E*)-3-hexen-1-ol (**8**), and (*Z*)-3-hexen-1-ol, although these volatiles show low sensitivity. (*E*)-3-Hexenal (**2**), which increases with ripeness (Figure 1), shows a great sensitivity, or ability to characterize this stage, but medium selectivity (possibility < 0.75).

From the results of this study it can be concluded that the unripe stage is the best characterized. This is a logical conclusion since we have quantified the volatiles responsible for the green odor note. Alcohols are basically responsible for the characterization because their values are rather apart from each other in the three stages of ripeness. However, hexan-1-ol (**7**) has such an attenuated filtered function that it would not be appropriate to characterize any stage. It is noteworthy that alcohols produced from the linolenic acid are the major contributors to the ripeness characterization, while hexan-1-ol, produced from linoleic acid, does not contribute at all. In the case of aldehydes, hexanal—produced from linoleic acid—is the major contributor.

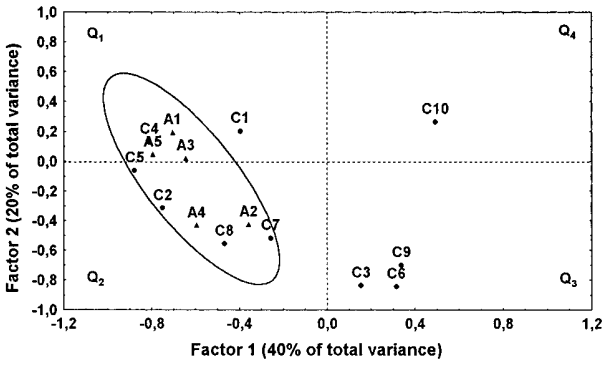

Figure 4. Two-dimensional plot showing the result of principal component analysis of volatile compounds and sensory attributes.

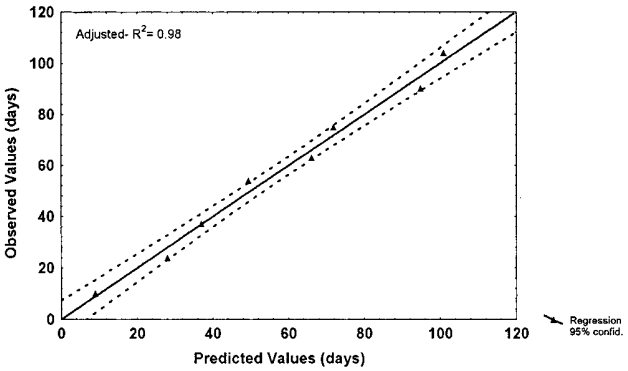

Figure 5. Results of the multiple regression applied to the test set of samples. *x*- and *y*-axes show the days of harvesting, the first day being October 1st. Observed values versus predicted values are plotted.

The volatile compounds characterizing the stages of ripeness were selected with the only criterium of their amount at each stage of ripeness. This does not mean that these volatile compounds contribute, more than the others, to green aroma evaluated by assessors; on the contrary, the characterization is usually carried out with minor compounds rather than major compounds as these change a few. On the other hand, the contribution of a volatile to aroma depends not only on its amount but also on its odor threshold in a matrix of deodorized oil. OAV (Grosch, 1994) is the parameter used to evaluate the contribution of volatiles to aroma, while sniffing is the habitual procedure to describe the volatiles from a sensory profiling. Table 2 shows the OAVs of volatile compounds. Five of 10 have OAVs >1, clearly contributing to the green aroma of olive oil. The other five volatile compounds, having green or fruity odor evaluated by HRGC-sniffing but OAVs <1, do not clearly contribute to the green aroma of olive oil by themselves, but their presence is important for its final global aroma.

From the information of Table 2 and Figure 3 we can infer that the characterization of the stages of ripeness depends on those volatiles with low values of OAV. As (*Z*)-3-hexenal and (*E*)-2-hexenal have the highest OAVs, they do not contribute to discriminate the stages of ripeness. This means the characterization of the stages of ripeness can be carried out by analytical quantification of volatiles rather than sensory profiling (Aparicio et al., 1994).

Previous studies using a sensory wheel (Aparicio and Morales, 1995; Aparicio et al., 1996b) or multidimensional scaling (Morales et al., 1995) showed that some of these volatiles, among others, were properly related with the zone of green attributes evaluated in virgin olive oil. Five attributes of this zone were selected for the subsequent analysis as they covered the whole range of green sensory notes: "olive fruity", "green fruity", "green", "banana", and "green-bitter".

The results of the volatile compounds analysis and sensory evaluation were analyzed by PCA. The total variance explained by three principal components was 71%. The first eigenvector explained 40%, the second 20%, the third 11%, and the remainder 19%. Figure 4 shows the resulting plot. The ellipse shows the confidence region of the green sensory notes selected from sensory panels. The volatile compounds inside the ellipse contribute more to green sensory perceptions than those outside.

Following the anticlockwise direction in Figure 4, quadrant Q1 clusters the attributes green fruity (A1), green (A5), and olive fruity (A3), which are placed close to hexyl acetate (C4) and hexanal (C1), indicating that quadrant Q1 represents the fruity green aspect of the overall green aroma. Attributes green-bitter (A4) and banana (A2) were placed inside the same quadrant (Q2) as (*Z*)-3-hexenal (C2), (*Z*)-3-hexenyl acetate (C5), (*Z*)-3-hexen-1-ol (C8), and (*E*)-3-hexen-1-ol (C7). This quadrant establishes a greater intensity of green aroma with a touch of astringency. Quadrant Q3 clusters hexan-1-ol (C6) and (*E*)-2-hexen-1-ol (C9)—both characterized as undesirable by the sensory wheel procedure and as astringent/bitter by tasting pure compounds—and (*E*)-2-hexenal (C3), which is characterized by its bitter almond odor. (*E*)-3-Hexenal (C10) appears alone inside quadrant Q4, indicating the different behavior of this compound versus the other ones (Figure 1) as it characterized the over-ripe stage of ripeness (Figure 3).

Once stated that the concentration of green volatile compounds is related to the ripeness of the olive fruit, and hence to olive oil green aroma, the test set of samples was analyzed to check the volatile compounds that better characterized the stages of ripeness using the fuzzy filter. Volatiles hexanal, hexyl acetate, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol were used.

The restrictive conditions imposed on the procedure, through *F*-to-enter (Tabachnick and Fidell, 1983), make the result of adjusted $R^2 = 0.98$ sufficiently reliable (Figure 5). The eight different stages of ripeness used (the samples were picked from the same orchard each fortnight for 4 months) guarantee that the quantitative values of these volatiles are a worthwhile alternative to other procedures based on visual inspection (EOC, 1976) in the determination of the stages of ripeness.

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