

Detection of Rancid Defect in Virgin Olive Oil by the Electronic Nose

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A sensor array of 32 conducting polymer sensors has been used to detect the rancid defect in virgin olive oils. A training set, composed of admixtures of a Portuguese virgin olive oil with different percentages (0–100%) of a rancid standard oil, was used for the selection of the best sensors classifying correctly the samples. Information on volatile compounds responsible for rancidity and the sensory evaluation of samples by assessors were used for explaining the mathematical selection of sensors. A tentative calibration, using unsupervised procedures (PCA and MDS) and a nonlinear regression, was carried out, with the training set, and later confirmed with a test set with which rancid commercial samples of different varieties were used to spike a Greek extra virgin olive oil at low levels of rancidity (0.5–6%).

Keywords: *Virgin olive oil; electronic nose; sensory evaluation; volatiles; rancidity*

INTRODUCTION

Virgin olive oil sensory quality is currently determined by the European Union regulation (EC, 1991) or the International Olive Oil Council trade standards (IOOC, 1996). Both official methods carry out the sensory evaluation by using panels of trained assessors, although there are certain technical differences between them, basically the kind of sensory descriptors and the scale of evaluation (structured versus nonstructured). These panel tests have been useful for the construction of a consensus between experts of different countries about which attributes would be present or absent in virgin olive oils of reputable quality. However, panel tests are a costly and slow procedure that is not always at the disposal of small producers or cooperative societies; only large retailers and sellers may be able to afford such tests. Furthermore, the subjective opinion of assessors undermines the final overall evaluation, and some flaws have been pointed out (Ranzani, 1994), mostly when multivariate procedures are not applied (Aparicio et al., 1992).

On the other hand, volatile compounds are responsible for the flavor perceptions detected by assessors, and they are not obviously subjective information but quite objective (Flath et al., 1973). On the basis of this fact, a recently proposed methodology correlates basic sensory descriptors (Aparicio et al., 1994; Aparicio and Morales, 1995) with the volatile compounds responsible for them (Morales et al., 1995; Aparicio et al., 1996). The methodology, to which the mathematical procedures give support (Aparicio and Morales, 1994; Morales et al., 1994), has allowed the explanation of the most remarkable virgin olive oil sensory descriptors (green, bitter–pungent–astringent, sweet, fruity, ripe fruit, ripe olives, and miscellaneous undesirable attributes).

However, this methodology requires quantification of the volatile compounds by dynamic headspace high-resolution gas chromatography (DHS-HRGC), which is today slow enough to be applied to on-line processes. The on-line control is a demand more and more heard from producers who want to store their olive oils in different deposits, according to olive oil quality, as soon as the oils have been produced from the automatic centrifugation systems. The importance is not then in the detection of those attributes responsible for high-quality virgin olive oils, for example, green and fruity (IOOC, 1996), but in the quantification of defects (Peri and Rastelli, 1994), rancidity being one of the most remarkable undesirable attributes.

As lipids oxidize, they form hydroperoxides, which are susceptible to further oxidation or decomposition to secondary reaction products such as aldehydes, ketones, acids, and alcohols. In almost all cases, these compounds adversely affect flavor, aroma, taste, nutritional value, and overall quality (Vercellotti et al., 1992). Because there are many catalytic systems that can oxidize lipids, for example, light, temperature, enzymes, metals, metalloproteins, and microorganisms, the control, quantification, and prediction of oxidation are still important issues from either scientific or economic points of view. From a chemical viewpoint, different methodologies have been suggested to measure and predict the oxidation (Gutiérrez, 1989; ISO 6886, 1989; Cabré and Massó, 1992; Morales and Aparicio, 1997). From a sensory point of view, a virgin olive oil is oxidized when assessors detect and quantify the presence of the rancid perception in the complex matrix of virgin olive oil (EC, 1991; IOOC, 1996).

A potential alternative, based on semiconductive organic polymers, has recently appeared for the evaluation of foods and food products (Persaud, 1991; Taylor et al., 1995; Dube and Peterson-Daly, 1996; Taylor, 1998; Tullett, 1996; Goldring, 1997; Visser and Taylor, 1998), although certain restrictions have been detected in their applications (Zannoni, 1995; van Ysacker and

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Ellen, 1998). The electronic aroma sensing system (Hermia and Vignerons, 1994; Grate et al., 1997; Persaud and Travers, 1997; Karube et al., 1997), or electronic nose (Bartlett and Gardner, 1992; Hermia, 1997) as the methodology is commonly called, has opened a new avenue for screening flavor compounds (Gardner et al., 1994; Hivert et al., 1995; Shiers and Farnell, 1995; Rocha et al., 1998). Thus, the concept of olfactometry, the human evaluation of volatile compounds at the sniffing port of a gas chromatograph, has abruptly changed with the arrival of sensors (Phillips, 1995). This paper analyzes the possibilities of these sensors in the detection of the rancid perception, one of the most remarkable undesirable attributes according to the International Olive Oil Council (IOOC, 1996), in high-quality virgin olive oil. This study goes beyond the results recently published about the application of sensors in the prediction of the shelf life of edible oils (Shiers and Aldechy, 1998). Thus, the relationships between the information given by sensors and the volatile composition of samples, sniffing, and their sensory evaluation have been studied. A calibration is carried out with the most noteworthy sensors.

MATERIALS AND METHODS

Materials. A rancid standard virgin olive oil of the International Olive Oil Council was used. This standard is currently used to train the assessors to identify and evaluate the rancid defect. The standard was added to a Portuguese virgin olive oil (*Olea europea* L.) at different percentages: 0, 1, 5, 12, 20, 40, 60, and 100%. The results were verified with a test set of 12 samples. These samples were made by adding different percentages (1, 5, 10, and 20%) of three virgin olive oils (varieties Frantoio, Italy, harvest 1992; Hojiblanca, Spain, harvest 1989; and Picual, Spain, harvest 1995) with clear rancid perception, according to assessors, to an extra virgin olive oil cv. Koroneiki (Crete, Greece, harvest 1997). The objective of the test set was not only to verify the results of the training set but also to check the detection of low levels of rancid perception in virgin olive oil, in comparison with the official sensory methodologies (EC, 1991; IOOC, 1996).

DHS-HRGC. Volatile compounds were analyzed with a dynamic headspace technique under determined optimized conditions as previously described (Aparicio and Morales, 1994; Morales et al., 1994). Samples of 0.5 g were heated at 40 °C and swept with N₂ (200 mL/min) for 15 min. A trap of Tenax TA (Chrompack) absorbed the volatiles at room temperature. A Chrompack thermal desorption cold trap injector (TCT) was used to desorb the trapped volatile compounds 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 made 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 DB-Wax capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific, Folsom, CA). The oven temperature was held at 40 °C for 6 min and programmed to rise at 2 °C/min to a final temperature of 200 °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. Volatile compounds were analyzed in duplicate.

Peaks were identified by mass spectrometry using conditions identical to those for gas chromatography. A Fisons MD800 mass selective detector coupled to a GC 8000 series (VG Analytical, Manchester, U.K.) was employed. Masslab v1.3 (VG Analytical, Manchester, U.K.) was the software used. Sample components were verified by comparison of mass spectral data with those of authentic reference compounds.

Gas chromatographic data were linked to a personal computer. ASCII files were manipulated to eliminate unwanted

information from the chromatographic reports by a Fortran program. The automated program performed the selection of peaks based on retention time ranges after visual recognition of a standard chromatogram. Retention time and areas of selected peaks, including the internal standard, were stored in a database (Ultrix/SQL, version 2.0). Ratios of each of the selected peak areas to the area of the internal standard were used for statistical analysis.

Sensory Properties of Volatile Compounds by Olfactometry. To assess the aroma notes corresponding to olive oil volatile compounds, an HRGC-sniffing technique was applied to virgin olive oil samples (Morales et al., 1995). 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 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 volatile compounds, although different semantic terms may have been used. A consensus-building discussion was held with assessors to decide the final sensory descriptors.

Sensory Analysis. The profile sheet of the International Olive Oil Council (IOOC, 1996) is divided into two types of sensory attributes, "positive" and "negative" (defects). The latter clusters sensory attributes that indicate defectiveness and even unpleasantness: fusty, mustiness, winey-vinegary-acid, muddy sediment, metallic, rancid, and others, whereas the former group clusters fruity, bitter, and pungent. The evaluation of attributes is made on an unstructured scale (IOOC, 1996). A mathematical program calculates the median from the graphical information given by assessors in the profile sheet.

An olive oil is then classified as extra virgin olive oil when the median of defects is 0 and the median of fruity is >0. The oil is classified as virgin when the median of defects is ≤2.5 but the median of fruity is >0. If the median of defects is >2.5 but <6.0 or if the median of defects is ≤2.5 but the median of fruity is 0, then the sample is classified as ordinary virgin olive oil. An olive oil is classified as lampant virgin olive oil when the median of defects is >6.0.

The samples of the test set were evaluated in triplicate by assessors following strictly the methodology suggested by the International Olive Oil Council (IOOC, 1996). The sensory analysis was carried out not only to detect the intensity of rancid defect in the samples but also to compare the limit of detection of this defect carried out by the assessors and sensors. This experiment is of great importance since some cherished sensory perceptions (e.g., fruity and green) can mask the rancid defect when the samples are evaluated with a panel test, whereas it is not expected when using sensors.

Sensors. An AromaScan A32S/8S Labstation System (AromaScan plc, Crewe, U.K.) was used in this study. This system comprises an analyzer (A32S) unit with a 32 element sensor array of conducting polymers and a sample station (A8S). The latter generates reference air of known quality and humidity that is used to fill the flask containing the sample. The equilibrated headspace is then pumped into the A32S analyzer and passed across the sensor array. The sensors are made of polymers that change their electrical resistance when a volatile compound adsorbs. The change in electrical resistance of each sensor element is measured, and all of the responses are converted into a normalized pattern of responses. Each sensor has a different characteristic response. The relative responses of individual sensors reflect the range of volatile compounds given off by a sample.

An initial tentative analysis was carried out to determine the optimum volume of the flask and the sample amount because there was no reference in the bibliography. The initial experiments were carried out with flasks of 10 mL with 1 and 3 mL of olive oil, but the response intensities of two sensors rose over time, pointing out that it was necessary to increase the amount of sample. After different experiments, the best results were obtained with 5 mL of olive oil inside flasks of

120 mL. The ratio of sample content to flask volume favors the surface contact between the liquid and gas phases, increasing the olive oil volatile compounds inside the headspace. All of the samples were stabilized to room temperature for 25 min inside hermetically closed flasks. The analyses were carried out at 25 °C and 6% humidity.

The following protocol for the valve sequence was established: reference 30 s, sample 120 s, wash 30 s, and reference 120 s. The reference consisted of 6% relative humidity air passed directly from the A8S sample station to the analyzer (A32S). The wash part of the cycle consists of passing over the sensor air that had previously been passed over a 2% water/butanol solution. The purpose of this part of the cycle is to avoid cross-contamination between successive samples. The effectiveness of the wash with butanol/water, for the reduction of the cross-contamination of the sensors, is controlled during the second reference step, when the response of the sensors has to return to zero. The sample station provides a wash (vapor) source through the wash line at the rear of the instrument. The wash source is generated from the headspace above the wash liquid (butanol/water 2%). The time of washing was 30 s because it allowed the return to zero. This protocol and the temperature and humidity controls guarantee that all of the measurements were carried out under identical conditions.

The information from the sensors was collected every 4 s during the interval 35–150 s. The methodology used to choose this interval was based on previous experiences (Rocha et al., 1998) where the behavior of the sensors was excellent. All of the samples were measured several times on different days. The samples were randomly selected for the analyses.

Mathematical Analysis. Principal component analysis (PCA) was applied to analyze the structure of data sets and detect abnormal information (outliers). Cross-validation (Martens and Naes, 1989), with different cancellation matrices, was used to detect the significant components for this study.

Stepwise linear discriminant analysis (SLDA) was applied to find the levels of correct classification of the samples by the information of sensors at different measurement times. The strictest conditions were applied to avoid the possibility of the results being obtained by chance. The criterion for variable (sensor) selection was the *F*-to-enter value (>5.05) obtained from the *F* distribution table (*F* = 0.95), taking into account the number of clusters (percentage of rancid standard in the virgin olive oil) and the minimum number of samples inside the clusters. An internal cross-validation was carried out with ~30% of the samples. The selection of samples for this internal test set was completely random.

Multidimensional scaling (MDS) was used to determine the inherent differences (multidimensional scaling is a nonsupervised procedure) among the samples spiked with the rancid standard at different percentages. A tree clustering classification algorithm, using a complete linkage amalgamation rule and city-block (Manhattan) distance (Shiffman et al., 1981), was applied to calculate the matrix of distances. The stress measurement was used to evaluate how well the final configuration reproduces the observed matrix of distances. A confidence ellipse was plotted for each natural group. The confidence area of the ellipses is based on the assumption that the two variables (roots) follow the bivariate normal distribution, and their orientation is determined by the sign of the linear correlation between two variables; the longer axis of the ellipse is superimposed on the regression line. The probability that the samples will fall within the area marked by the ellipse was fixed to 0.95.

The goal of canonical correlation (CC) was to analyze the relationship between two sets of variables (volatile compounds and sensor responses) to see how the two sets relate to each other. Selection of pairs of canonical variates was made by Bartlett's test.

Regression on principal components (RPCA), which maintains the idea of the standard multiple regression procedure but with orthogonal variables (the principal components), was applied to correlate the sensor responses and the percentages of rancid standard in virgin olive oil sample. Ridge regression

Table 1. Volatile Compounds Mainly Responsible for Rancid Defect Characterized by Sniffing (Olfactometry)^a

volatile	sniffing	content (ppm)	
		standard	VOO
pentanal	woody, pungent	13.04	0.015
hexanal	powerful green, fatty	132.25	0.365
heptanal	fatty, woody	7.12	0.027
2-heptenal	soapy, tallowy	9.98	0.025
nonanal	waxy, painty	27.82	tr
acetic acid	pungent, sour	8.67	0.026
2,4 heptadienal	fatty, rancid	1.82	0.085
decanal	soapy	1.50	0.005
2-decenal	tallowy, soapy	4.61	tr
nonanol	fatty	1.52	tr
2,4-nonadienal	soapy, penetrating	0.75	tr
butanoic acid	rancid	7.30	tr
undecanal	pungent	2.59	0.006
2,4-decadienal	fatty	1.56	tr
hexanoic acid	rancid, pungent	25.33	0.007
heptanoic acid	rancid	0.87	0.004

^a The contents of volatile compounds, in the rancid standard and the mean of 87 European VOOs, are referred to the virgin olive oil headspace under the conditions described under Materials and Methods.

(RR) was applied to analyze the relationship between volatile compounds and the response of sensors. This regression procedure was applied because high values of correlation between sensors were detected.

Statistica (Statistica, 1995) and BMDP (Dixon, 1983) were used to implement the statistical procedures of PCA, SLDA, MDS, CC, RPCA, and RR.

RESULTS AND DISCUSSION

Table 1 shows the most representative chemical compounds identified in the rancid standard from a large set of volatile compounds (Morales and Aparicio, 1997). The table also shows the mean of these compounds in 87 extra virgin olive oils from different countries and varieties (Morales et al., 1995; AIR, 1998) and the content of these compounds in the rancid standard. There are obviously clear differences between the profile of virgin olive oils (VOO) and this standard. However, the most remarkable is the number of volatile compounds that are not detected in VOO but in the standard, pointing out the possibility of detecting these sensory attributes in VOO even at very low intensities.

Column 2 ("sniffing", Table 1) shows the sensory characterization of each volatile. This information confirms that the selection of volatile compounds has a sensory explanation for the rancid defect. The sensory characterization of each volatile by sniffing is of great interest because the correlation between sensors and volatiles can allow characterization of the former ones in terms of sensory quality. This characterization can also be useful to check whether the array of selected sensors procures a sensory profile similar to that given by assessors. Anyway, the volatile with which a sensor is correlated should agree with the sensor sensitivity to the volatile.

In general, there are two main concepts used in the measurement of off-odors: detectability and intensity. To study these criteria, the samples of the training set, constituted by the addition of rancid standard to a Portuguese VOO at different percentages (0, 1, 5, 12, 20, 40, 60, and 100%), were evaluated by a sensor array of 32 sensors. The sensor array has sensors sensitive to alcohols of short and long chains, carboxylic acids, aromatics, chlorinated hydrocarbons, esters of short and

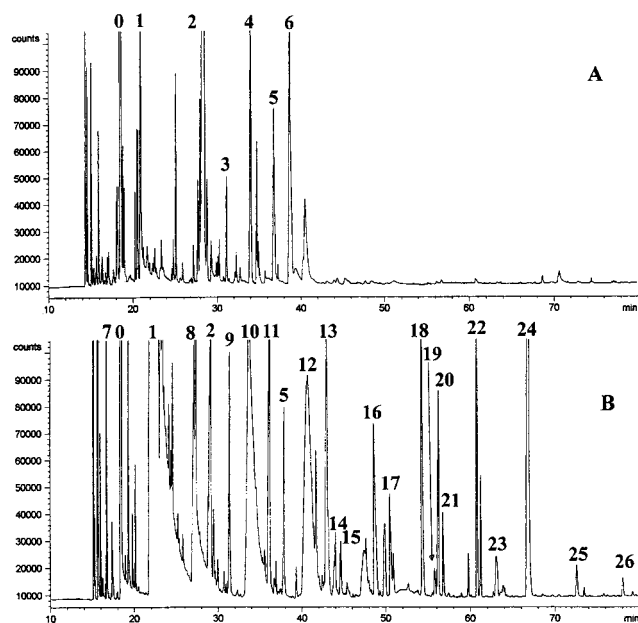


Figure 1. Chromatograms of VOO (A) and the rancid standard (B). The amount of sample of rancid standard was half that of VOO. Peaks: 0, internal standard; 1, hexanal; 2, (*E*)-2-hexenal; 3, hexyl acetate; 4, penten-2-ol; 5, hexan-1-ol; 6, (*Z*)-3-hexen-1-ol; 7, pentanal; 8, heptanal; 9, 1,3-nonadiene; 10, octanal; 11, 2-heptenal; 12, nonanal; 13, acetic acid; 14, 2,4-heptadienal; 15, decanal; 16, propanoic acid; 17, undecanal; 18, butanoic acid; 19, 2,4-nonadienal; 20, 2-decanal; 21, nonanol; 22, pentanoic acid; 23, 2,4-decadienal; 24, hexanoic acid; 25, heptanoic acid; 26, octanoic acid.

long chains, ketones plus water, and amines. The importance of including sensing materials with diverse properties is of great importance as the sensor array will best spread out a diverse set of vapors and hence facilitate discrimination. However, it is important to examine the data generated by an array of odor sensors so that it can be possible, for example, to identify sensors which clearly generate small and noisy signals, to detect sensors that exhibit a high degree of linearity with other sensors, to identify spurious responses, etc. To study these and other aspects, the analysis of data was carried out using the following protocol: (i) detection of outliers (samples); (ii) study of repeatability of the analyses; (iii) selection of the measurement interval; (iv) selection of the best sensors for rancid defect; (v) evaluation of the discriminative capacity of sensors; and (vi) evaluation of a possible calibration. Finally, the calibration equation was applied to a test set of samples spiked at percentages even lower than used in the training set.

PCA was used to detect abnormal data (outliers) (Tabachnick and Fidell, 1983) in the studies described below. Five outliers were detected and removed from the data set. Two of them were detected when replicates of the rancid standard were analyzed, whereas the others were detected by evaluating replicates of the samples spiked with the official standard at 60 and 40%.

Each of the samples was analyzed various times at different hours and days to study the repeatability of the analyses and detect the influence of ambient conditions (humidity and temperature) on the measurements. The coefficient of variation oscillated between 2.5% for the Portuguese VOO and 12.7% for the rancid standard. Figure 1 shows the chromatograms of the Portuguese VOO and the rancid standard (oxidized oil). There is a great difference between their profiles, acids and aldehydes being remarkably higher in the oxidized sample.

An analysis of the response of the sensors to iterative analysis of the same sample showed that the highest coefficients of variation (CV) corresponded to the first results of the experiment and slightly to the last ones. The CVs of the samples, analyzed one by one, oscillated between 2 and 9%, the highest value corresponding to the rancid standard. These figures indicate that some sensors could have been close to saturation due to the great amount of chemical compounds in the rancid standard.

It is well-known that the sensors need a certain period of time to give a response to the excitation produced by the volatile compounds (Rocha et al., 1998). An SLDA was used to detect the best interval of time differentiating the samples spiked with the standards. SLDA was applied with *F*-to-enter selected from an *F* table at 0.95%, taking into account the number of samples and variables (sensors). The response of sensors in the interval 34–64 s was too poor up to the point that the discrimination was impossible. This interval was removed, and the following studies were based on the interval 69–120 s.

Once the interval of time was selected, the mean of the raw intensities of each sensor during the interval was calculated. Figure 2 shows the mean of the raw responses of the sensors to the rancid defect during the selected interval. The figure shows that there were sensors with the ability to distinguish VOO from the spiked samples and to classify the samples at the addition percentages (0, 1, 5, 12, 20, 40, 60, and 100%). These sensors were numbered 1, 5, 11, 12, 13, 27, 30, 31, and 32. These sensors, however, showed a behavior contrary to that expected. Their responses were lower for the rancid standard and spiked samples than for VOO, despite the amount of volatile compounds being lower in VOOs (Morales and Aparicio, 1997). It could be suggested that this is distinctive of a difference of humidity between the fresh sample and the rancid standard. A very small difference, e.g., 700 versus 900 ppm, may lead to an equivalent response of hundreds of parts per million of volatile due to the higher sensitivity of conducting polymers to water. However, this behavior can also be explained by the increase of their resistance, due to polymer swelling, when adsorbing the volatile compounds. The last hypothesis seems to be more plausible as the analyses were carried out at 25 °C and 6% humidity on different days, and at various times, with a good repeatability.

An RR analysis of the sensors with each of the volatile compounds (Table 1) showed that the regression coefficient oscillated between $R = 0.72$ and $R = 0.79$ ($p < 0.05$). The volatile compounds correlated with the sensors were the alcohol nonanol (5, 10, 12, 13, 27, 30, 31), acetic (1, 19), heptanoic (21), and butanoic (11, 32) acids, and the aldehyde pentanal (10). All of the selected sensors were correlated with those volatile compounds to which they were sensitive, with the exception of sensor 10, which was correlated with a C_5 aldehyde. On the other hand, the profile of volatiles is responsible for the rancid perception, and hence the correlation (CC) between the data sets of volatiles and sensors would indicate how useful the sensors are in emulating the whole profile of volatiles. Thus, when CC was applied to both sets of data (volatiles and sensors), the regression coefficient was low enough ($R = 0.73$). This coefficient would have been higher whether had there been sensors sensitive to aldehydes of short chain (C_6 – C_9),

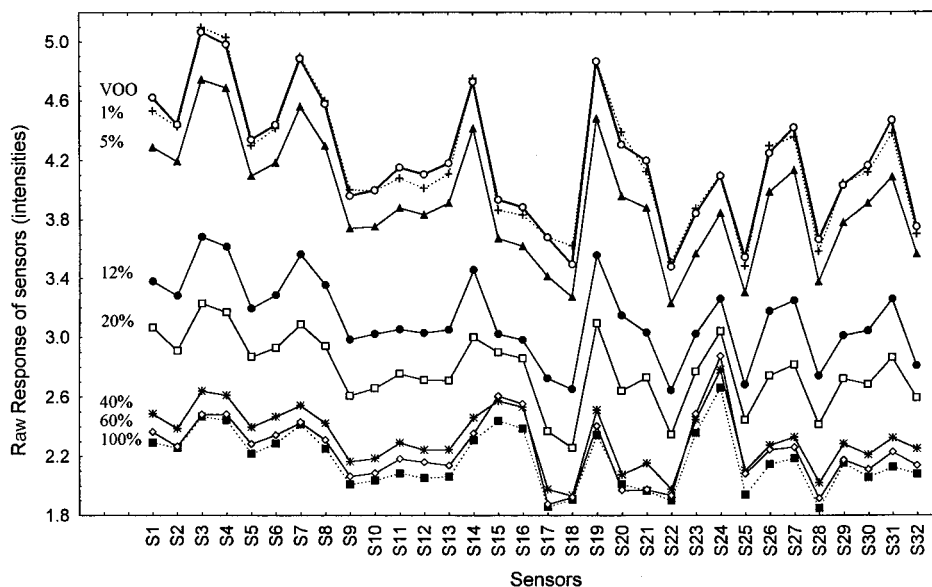


Figure 2. Mean of the raw response of the sensors (time between 69 and 120 s) to a VOO and samples spiked with different percentages of the rancid standard.

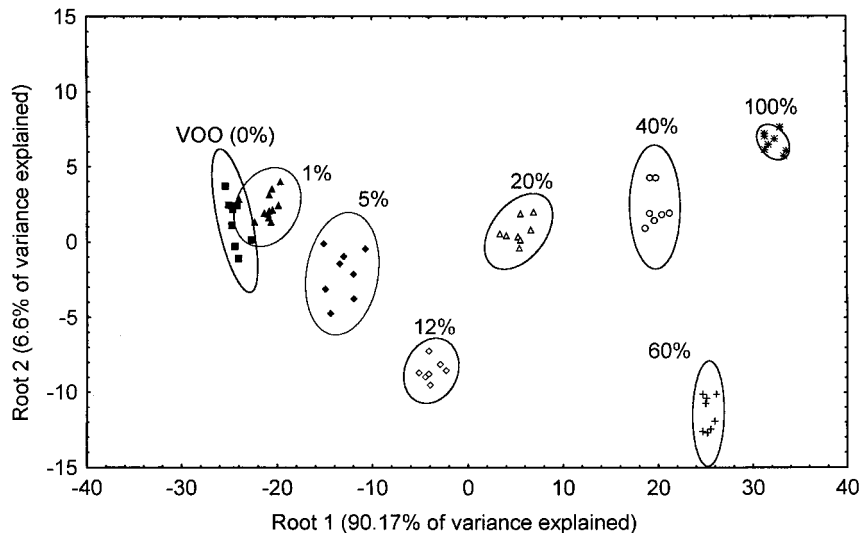


Figure 3. Results of applying MDS. The ellipses of confidence were plotted at a probability of 0.95.

which are the major compounds in oxidized oils (Frankel, 1985). Morales and Aparicio (1997) have stated that the quantification of hexanal and nonanal is enough for detecting oxidized VOO even before a panel test could quantify the attribute rancid.

The sensory characterization (sniffing) of the volatile compounds correlated with the selected sensors can allow hypothesizing about the sensory profiles of samples from the point of view of volatiles and, hence, of their correlated sensors. However, the content of the compounds in the samples varies according to the percentage of rancid standard added to VOO and, on the other hand, volatile compounds have different levels of detection (odor thresholds) for assessors. If the content of a chemical compound is lower than the odor threshold, then this compound will not individually contribute to the flavor, as assessors would not detect it. However, it can also be argued that it can contribute to the flavor due to possible phenomena of synergy/enhancement with other compounds. Table 2 shows the odor thresholds (parts per million) of the selected chemical compounds calculated in paraffin oil. Thus, the ratio between the content of the volatile compounds in the

Table 2. Threshold Values of Volatile Compounds Responsible for Off-Flavor Components

volatile	odor threshold	volatile	odor threshold
pentanal	0.24 ^a	2-decanal	
hexanal	0.32 ^a	nonanol	0.28 ^d
heptanal	3.21 ^b	2,4-nonadienal	
2-heptenal		butanoic acid	0.14 ^c
nonanal	13.5 ^a	undecanal	
acetic acid	0.50 ^d	2,4-decadienal	2.15 ^a
2,4 heptadienal	3.62 ^a	hexanoic acid	0.70 ^d
decanal	6.72 ^a	heptanoic acid	0.10 ^d

^a Odor threshold values (mg/kg) in paraffin oil obtained from Meijboom (1964). ^b Odor threshold values (mg/kg) in paraffin oil obtained from Kochhar (1993). ^c Odor threshold values (mg/kg) in sunflower oil obtained from Grosch (1994). ^d Odor threshold values (mg/kg) in refined VOO.

rancid standard and their odor threshold indicates that assessors would not detect heptanal in admixtures lower than 45% (threshold = 3.21 ppm; content at 45% is 3.20 ppm) or nonanol in admixtures lower than 18% (threshold = 0.28 ppm; content at 18% is 0.27 ppm), respectively. Heptanoic, acetic, and hexanoic acids would be

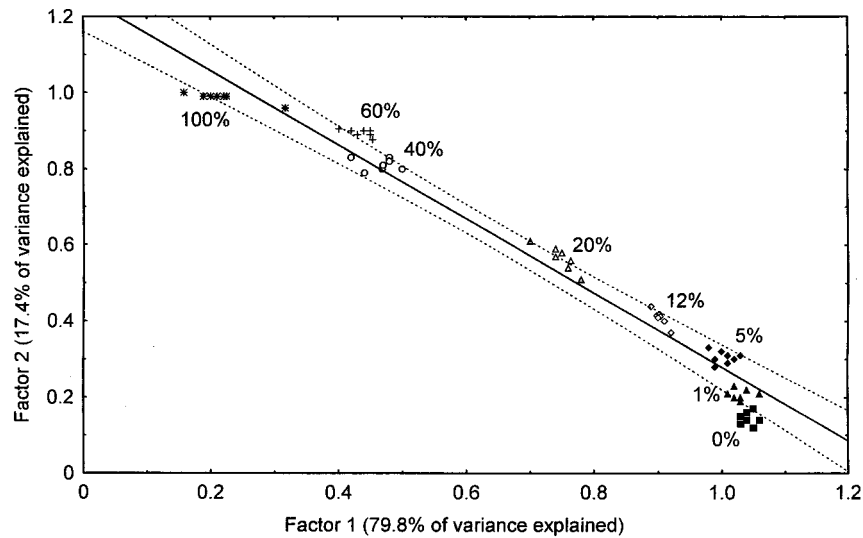


Figure 4. Result of the RPCA. The signs of each group represent the measurements carried out with each sample (0, 1, 5, 12, 20, 40, 60, and 100% of the rancid official standard) on different days. The abnormal data were previously removed.

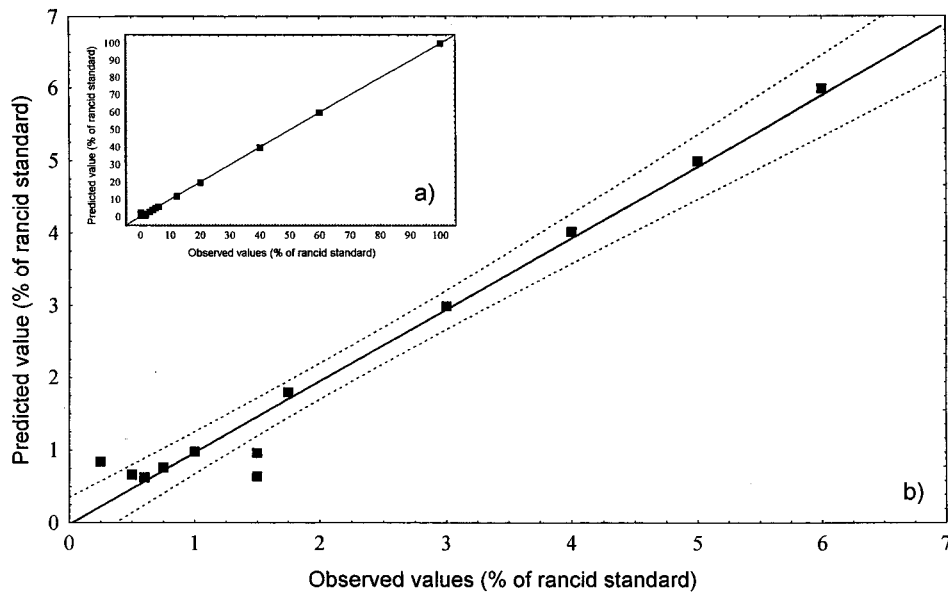


Figure 5. Calculated rancid defect (percent) in the test set of samples determined by applying the nonlinear regression equation of the training set: (a) projection of the test set results on the regression of the training set; (b) magnified results of the test set.

detected in admixtures above 12, 6, and 3%, respectively, whereas the aldehydes 2,4-heptadienal and 2,4-decadienal would not contribute to the rancid sensory perception. From an analysis of the other volatile compounds, pentanal and butanoic acid would contribute to the detection of rancid perception in admixtures above 2%. The case of hexanal is peculiar because it is present in the initial VOO as it is produced from linoleic acid through the lipoxygenase pathway. The authors (Aparicio et al., 1996) have demonstrated that hexanal is an important flavor compound of VOO, and it has been positively correlated with the overall acceptability of potential and habitual consumers of VOO for values in the range of 0.03–1.06 ppm.

A panel test (IOOC, 1996) evaluated samples of Portuguese VOO spiked with the rancid standard at different percentages (0, 1, 5, 10, 12, 20, 40, 60, and 100%). The assessors decidedly detected the rancid defect when the addition was $\geq 10\%$ and classified the sample as lampant-VOO. The addition of the rancid standard at 5% was very slightly detected as the strong green-fruity perception masked the defect, and hence

the assessors classified the samples as ordinary virgin olive oil. The assessors had more problems detecting the presence of the rancid defect at 1%, and the sample was classified in the neighbors of VOO.

The next step was to analyze up to which point the selected sensors were able to discriminate among the samples. The results of Figure 2 promised good results, and in fact Figure 3 shows that the unsupervised statistical procedure of MDS (Shiffman et al., 1981) was able to distinguish one by one the samples spiked with the official standard. The ellipses of confidence, plotted for each group inside the figure, show that there is no overlapping between the groups. An analysis of the variance explained by each root (90.2 versus 6.6%) shows that the groups are explained mostly by the first root, and their position in relation to it goes from zero addition of the rancid standard to 100%. This means that there is a certain possibility of calibration of the array of sensors with respect to the rancid perception. Regression on principal components was the statistical procedure used for a tentative calibration of the rancid perception.

Table 3. Rancid Samples (Test Set) of Varietal Olive Oils (Frantoio, Hojiblanca, and Picual) Added to an Extra Virgin Olive Oil of Koroneiki Variety at the Percentages Described in the Third Column^a

code	rancid sample	addition (%)	peroxide value	hexanal to nonanal ratio	rancid defect (%)
I1	Frantoio	20	8.8	4.1	5.99
I2	Frantoio	1	7.5	8.8	0.76
I3	Frantoio	10	8.5	4.9	2.99
I4	Frantoio	5	8.3	6.5	1.79
G1	Picual	20	8.5	3.1	4.99
G2	Picual	5	8.2	3.5	0.66
G3	Picual	1	7.7	4.4	0.64
G4	Picual	10	7.8	4.8	0.98
E1	Hojiblanca	20	8.3	4.3	4.01
E2	Hojiblanca	5	8.2	6.0	0.83
E3	Hojiblanca	1	7.8	9.7	0.62
E4	Hojiblanca	10	8.2	5.6	0.96

^a Peroxide value and hexanal/nonanal ratio of the test set samples. Percentage of rancid defect (in terms of rancid standard) calculated by the nonlinear regression procedure.

Figure 4 shows that the results are fine, although there is a certain nonlinearity in the sensor response, suggesting that a quadratic fitting could be better (Gardner and Hines, 1997). In fact, the use of a linear regression on nonlinear sensors is adequate if we accept that a relevant part of the nonlinear response space (0–12 and 60–100%) may be approximated by a linear region. Anyway, when covering the whole region of the response space, the sensors have a nonlinear concentration response, and so a nonlinear technique is required. A nonlinear regression based on an exponential growth equation [$y = c + \exp(b_0 + b_1x_1 + b_2x_2 + \dots)$] with a quasi-Newton estimation method and a convergence criterion of 0.01 was applied to the selected sensors (1, 5, 11, 12, 13, 27, 30, 31, 32). The equation explained 0.97 of the variance, whereas the regression coefficient was 0.986.

Between the two sources of nonlinearity described above (0–12 and 60–100% of rancid perception), the detection of rancidity in samples with very low rancid perception is of greater interest. A sample with a high level of rancidity is not fit for consumption as it is, and the sample must be refined. However, a sample with a low level of rancidity can lead to its classification as (extra) virgin olive oil, instead of ordinary virgin olive oil (EC, 1991), with the consequent profit. The test set was used to analyze this region. Figure 5 shows that the samples were classified between 0.6 and 6%, in terms of the rancid standard (100%), using the nonlinear regression based on the responses of sensors (1, 5, 11, 12, 13, 27, 30, 31, 32) to the training set. These results basically agree with the classification of samples by the hexanal/nonanal ratio (Morales et al., 1997), whereas the peroxide value does not show substantial differences between samples (Table 3). Assessors also evaluated the samples, but they were unable to order them correctly, although the rancid perception was detected at percentages >3%. Assessors rejected the admixtures >3% for consuming and had certain doubts about the samples between 0.6 and 3% that were characterized basically as "painty, pungent (penetrating), woody". If we suppose that the selected sensors had been assessors, then the sensory profile of the samples had been characterized as "fatty" for admixtures <2%, "fatty, woody, pungent, rancid" for admixtures between 2 and 12%, and "sour, waxy, and painty" for admixtures >50%. The fine results of the array of conducting polymer sensors can be due to the fact that they detect

the concentration of volatile compounds, and hence they do not have the limitation of their odor threshold that assessors have.

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