Analysis of Volatile Markers for Virgin Olive Oil

Aroma Defects by SPME-GC/FID: Possible

Sources of Incorrect Data

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ABSTRACT

The need to explain virgin olive oil (VOO) aroma descriptors by means of volatiles has raised interest in applying analytical techniques for trapping and quantitating volatiles. Static headspace sampling with solid phase microextraction (SPME) as trapping material is one of the most applied solutions for analyzing volatiles. The use of an internal standard and the determination of the response factors of the main volatiles seem to guarantee the correct determination of volatile concentrations in VOOs by SPME-GC/FID. This paper, however, shows that the competition phenomena between volatiles in their adsorption to the SPME fiber, inherent in static headspace sampling, may affect the quantitation. These phenomena are more noticeable in the particular case of highly odorant matrices, such as rancid and vinegary VOOs with high intensity of defect. The competition phenomena can modify the measurement sensitivity, which can be observed in volatile quantitation as well as in the recording of internal standard areas in different matrices. This paper analyzes the bias of the peak areas and concentrations of those volatiles that are markers for each sensory defect of VOOs (rancid, vinegary, musty, and fusty) when the intensity and complexity of aroma are increased. Of the 17 volatile markers studied in this work, 10 presented some anomalies in the quantitation in highly odorant matrices due the competition phenomena. However, quantitation was not affected in the concentration ranges at which each volatile marker is typically found in the defective oils they were characteristic of, validating their use as markers.

Keywords: Virgin olive oil, volatile compounds, sensory defects, SPME-GC, competition phenomena.

1. INTRODUCTION

The sensory characteristics of virgin olive oil (VOO) categories (extra virgin, virgin, ordinary, and lampante) are based not only on the detection of desirable sensory notes (fruity, apple-like, grass, bitterness, pungency, etc.) in the oil sample but mainly in the detection of undesirable sensory notes or sensory defects at different intensities. The four most common off-flavors are fusty, mustiness–humidity, winey–vinegary, and rancid. The first three defects are due to inadequate fruit preservation before olive oil extraction, whereas the last is produced during olive oil storage.(1-3) Thus, a sample is qualified as extra virgin olive oil (EVOO) when experienced panelists do not detect any of these sensory defects and it also has a detectable fruity attribute, whereas it is just VOO when a slight intensity of some sensory defect is detected by the panelist.(4, 5) The standard regulations(4, 5) describe how the sensory assessment has to be carried out by trained panelists. However, this procedure is not exempt from drawbacks due to external variables and factors resulting, at times, in wrong classification of VOO.(6) The key point is the difficulty of detecting sensory defects at very low intensities that sometimes are unnoticed.

The defects are perceived by panelists because of the presence of volatiles, from diverse molecular weight and chemical nature,(3) at concentrations higher than their odor thresholds.(2, 7) The current malaise of olive oil actors with some incongruence in qualifying VOOs is resulting in a demand for a new method quantitating the volatile compounds that are responsible for VOO off-flavors to support the current official sensory assessment.(8) In this context, the European research program Horizon 2020(9) stated that there is a need for the development, validation, and pre- as well as conormative activities followed by the standardization of a method for the assessment of the organoleptic characteristics of VOO. Continuing with this approach, several analytical methods have been proposed considering

studies carried out over years and existing knowledge on the volatile markers for each sensory defect.(2, 10-12)

Today, the most used analytical method for quantitating volatiles in VOOs is based on their concentration in a solid phase microextraction (SPME) fiber and subsequent desorption into a gas chromatograph (SPME-GC). This procedure of sampling volatiles has the advantages of being a solvent-free procedure that is cost-efficient and easy to implement at any laboratory and allows automation.(13, 14) On the other hand, the preconcentration of volatiles with SPME is not free of analytical drawbacks, including poor selectivity and low efficiency for some compounds among others.(15, 16) However, the main inconvenience is the competition and interaction phenomena during the adsorption process of volatile compounds onto the fiber.(13) These phenomena have not been studied in a lipid matrix yet, although they have been described in general terms.(17) The sensitivity of the SPME-GC method depends on the distribution of volatile compounds partitioned between the sample volume, the headspace volume, and the fiber coating.(18) Thus, the extraction of volatiles by SPME adsorbent is affected by the affinity of compounds for the fiber and the double equilibrium between the oil and the vapor phase and between the latter and the SPME adsorbent material. Considering the two equilibria, the extraction efficiency is defined by the phase ratio (β),(13) establishing the relationship between the volumes (V) of the VOO sample and the adsorbent material:

$$\beta = (V_{\text{virgin olive oil}}/V_{\text{SPME adsorbent}})$$

Given that this ratio is quite high due to the small volume of the SPME adsorbent coating, some variance may be observed in the recovery factors due to the presence of volatile compounds at high concentrations that may compete with others in the adsorption to the fiber. This competition phenomenon may be more significant when samples with a complex aroma are analyzed, where there are many volatile compounds at high

concentrations and a compound with a high affinity to the coating may displace another with less affinity(13) and where the quantitation of compounds that are present at trace level could be compromised. Competition phenomena may be observed as a lack of linearity/sensitivity, and they may have an impact on quantitation.(17) It is important to note that some of those compounds may have an important sensory contribution because of their low odor thresholds, despite their low concentrations.

Notwithstanding the problem stated above, the SPME-GC technique is still a practical tool that allows obtaining chemical information on food aroma with enough sensitivity and is amenable for being proposed as a support method for sensory assessment of virgin olive oil. A set of volatile markers has been already selected for each sensory defect.(2, 10) Because the European research program Horizon 2020 has encouraged the development of a support method for the assessment of the organoleptic characteristics of VOO, prior to suggesting any method based on SPME, a study of the effect of the competition phenomena on the quantitation of volatile markers of VOO is required. Thus, the objective of this work is to examine the adsorption process of volatiles of VOO onto the SPME fiber at different concentrations and complexities of aroma profile and also to check the competition and interaction phenomena over quantitation. The work allowed understanding how the static headspace sampling affects the preconcentration step, resulting in linear and nonlinear responses of the areas and the concentrations of volatiles depending on the complexity of the VOO matrix in terms of amount and diversity of its volatiles. The knowledge gained in this study allows checking whether the great diversity of intensities and kinds of aroma of VOO affects the volatile quantitation.

2. MATERIALS AND METHODS

2.1. Samples

Two monovarietal EVOOs (var. Picual and Hojiblanca) and four standards of VOOs qualified with sensory defects, supplied by the International Olive Council (IOC) and henceforth named reference defect samples, were used to obtain mixtures of different intensities of each defect. These four reference oils were characterized with the following negative attributes and medians of the defect intensity (Md, in scale of 10 points): rancid (8.65), fusty (5.70), musty (4.65), vinegary (8.50). Mixtures were prepared by spiking each monovarietal EVOO (var. Picual and Hojiblanca) with increasing percentages of each of the reference defect samples (5.0, 10.0, 17.5, 25.0, 37.5, 50.0, 67.5, 75.0, and 87.5%). The unspiked monovarietal EVOOs and the reference defect samples were associated with 0 and 100%, respectively.

2.2. Chemicals

Octane, ethyl acetate, pentanal, hexanal, heptanal, octanal, nonanal, (E)-2-heptenal, heptan-2-ol, 1-octen-3-ol, 1-octen-3-one, acetic acid, propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, and heptanoic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Determination of volatiles

The determination of volatiles was carried out according to the method applied in previous works.(19, 20) An olive oil sample (2 g) spiked with 2.6 mg/kg 4-methyl-2-pentanol (internal standard, IS) is placed in a 20 mL glass vial, tightly capped with polytetrafluoroethylene (PTFE) septum, and left for 10 min at 40 °C for equilibration of the volatiles in the headspace. After the equilibrium time, the septum covering each vial is pierced with a SPME needle, and the fiber is exposed to the headspace for 40 min at 40 °C. The fiber is then inserted into the injector port of the GC.

The concentration step was carried out on a Combipal (CTC Analytics AG, Zwingen, Switzerland). The temperature and time were automatically controlled by the software Varian Star Chromatography Workstation v6.41 (Varian, Walnut Creek, CA, USA). The SPME fiber (1 cm length and 50/30 μm film thickness) was purchased from Supelco (Bellefonte, PA, USA), and it was endowed with the Stable Flex stationary phase of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). The fiber was previously conditioned following the instructions of the supplier.

The volatiles adsorbed by the fiber were thermally desorbed in the hot injection port of a GC for 5 min at 260 °C with the purge valve off (splitless mode) and deposited onto a TR-WAX capillary column (60 m $\times$ 0.25 mm i.d., 0.25 coating; Teknokroma, Spain) of a Varian 3900 gas chromatograph with a flame ionization detector (FID). The carrier gas was hydrogen, at a flow rate of 1.5 mL/min. The oven temperature was held at 40 °C for 10 min and then programmed to rise at 3 °C/min to a final temperature of 200 °C. The signal was recorded and processed with the WorkStation (v6.41) software. Each sample was analyzed in duplicate.

The identification of the volatiles was carried out by chemical standards,(2, 10) which were purchased from Sigma-Aldrich. Table 1 shows the volatile markers of sensory defects considered in this study.

2.4. Response factor

Concentrations in the ranges of 0.1–5.0 and 0.5–30 mg/kg were analyzed under the analytical conditions described above. These two ranges allowed analyzing the recovery of volatile compounds at two different concentration levels. The absolute response factors of the standard compounds were calculated as the slopes of the linear regressions obtained from the ratio of total peak area as a function of concentration (averaged value from the two studied

ranges). Relative response factors were obtained as the ratio of the absolute response factor of each compound to that of the internal standard (4-methyl-2-pentanol).

2.5. Sensory evaluation by assessors

Fifteen panelists smelled the oil samples (5 g) from opaque vials (25 mL) to avoid the influence of the color in the decision. The vials were closed for >40 min prior to being tested. Panelists, who had several years of experience, were trained according to the International Olive Council regulation.(21) This training was based on the perception of the most common individual flavors and off-flavors present in virgin olive oil even at low intensity.

The sensory evaluation in the present work was carried out following a procedure partially inspired by the IOC regulations for sensory assessment.(21) The standard procedure for qualifying VOOs, which is based on a quantitative–descriptive test (so-called panel test),(4) has the exclusive objective of classifying VOOs into categories.(4) However, our main objective was to know the acceptability or rejection of VOOs by panelists.(22) Thus, the sensory test has to be focused on determining the global acceptability of VOOs as a consequence of the interaction between volatile compounds rather than detecting specific sensory defects or appreciated flavors as in the panel test. In the programmed sensory study, the scale for determining the opinion has to be hedonic, instead of the unstructured scale of the panel test, to make easier the understanding of the global perception by panelists.

The sensory evaluation by trained panelists was focused on the determination of the level of rejection to consume VOOs qualified with different levels of intensity of the most common off-flavors: fusty, musty, rancid, and vinegary. Panelists were asked not only about the sample preference but also about the sample consumption using a simplified hedonic scale. The hedonic scale used to measure the degree of pleasantness of a given product was committed to verbal categories such as extremely, very much, moderately, and slight (here

associated with “I would consume the oil”); neither like nor dislike (here associated with “I may consume the oil”), and dislike slightly, dislike moderately, dislike very much, dislike extremely (here related to “I would not consume the oil”). Each category was associated with a score, as in the scale developed by Peryam and Pilgrim.(22) After smelling each sample, panelists wrote one of those options that would better fit their expectations in terms of consuming the evaluated VOO.

The sensory evaluation was carried out in two steps. In the first step, the assessors qualified five additions (0, 10, 25, 50, and 100%) of each sensory defect (fusty, musty, rancid, and vinegary) to each EVOO. Once the rejection threshold of each mixture was determined, in a second step, the assessors evaluated additions with percentages that were lower and higher than the rejection threshold for a finer determination of the rejection threshold. The rejection thresholds for each sensory defect mixed with each of the EVOOs (Hojiblanca and Picual varieties) corresponded to the percentages of the defect sample at which 60% of the panelists rejected the mixed sample to consume it. The determination of the threshold was also inspired by the IOC regulation.(21)

2.6. Statistical Analysis

Linearity assessment was done by applying Ridge Regression (RR). This procedure is suitable when the independent variables are highly interrelated.(23) The performance of the regression was expressed with adjusted squared coefficient (R^2_{adj}). All of the statistical analyses were carried out with Statistica 6.0 (Statsoft Inc., Tulsa, OK, USA).

3. RESULTS AND DISCUSSION

The study of the competition phenomena between volatiles in static headspace sampling could have been carried out by adding standards of each volatile compound to EVOO var. Hojiblanca and var. Picual. However, the resulting volatile profile following this

procedure is not representative of a real sample with a complex aroma. Thus, we opted for using VOOs being reference samples of each sensory defect (rancid, vinegary, musty, and fusty) supplied by the IOC instead of pure compounds. These reference samples are characterized by a complex aroma produced by all of the volatiles responsible for each sensory defect (henceforth markers).⁽²⁾ Table 1 shows the volatile compounds that are markers of the main sensory defects according to previous studies,⁽²⁾ which were carried out with a Tenax TA trap with a dynamic headspace sampling. These volatiles were selected as markers of sensory defects because they are at low concentrations or absent in EVOO and because their odor thresholds are notably lower than their concentrations and, therefore, they contribute to the aroma perceived by consumers and panelists.

To obtain samples with an increasing level of complexity in their aroma profile, the reference defect samples were added to EVOO (var. Picual and Hojiblanca) at rising percentages (0, 5.0, 10.0, 17.5, 25.0, 37.5, 50.0, 67.5, 75.0, 87.5, and 100%). Those samples were consequently characterized by a rising intensity of defects. Table 2 shows the rejection threshold expressed as the percentage at which 60% of the panelists rejected the oil for consumption. In most of the cases that rejection threshold was 37.5%. However, in rancid and fusty mixtures prepared with EVOO var. Hojiblanca, the panelists rejected the oil at a lower percentage of added reference defect sample. The masking and synergic effect between compounds in their sensory perception could explain why the mixtures with EVOOs var. Hojiblanca and Picual yielded two different rejection thresholds. On the other hand, the rejection threshold was particularly high for musty mixtures prepared with EVOO var. Hojiblanca (75%). This high threshold is explained by the low content of volatile compounds and low odor intensity of musty VOOs,⁽²⁾ which make necessary adding a greater amount of musty reference sample to be perceived.

All of the mixtures were analyzed by SPME-GC/FID. The apparent concentrations of the volatile markers cited in Table 1 were expected to be higher as the percentage of reference defect samples increases in the resulting mixture. On the other hand, ideally the concentration value should exhibit a linear relationship with the percentage of added reference defect sample. Any observation of nonlinearity behavior can be due to a nonlinear response of the method, which was already studied with standards of volatile compounds diluted in refined olive oil.(10) However, the occurrence of many volatile compounds in the oil due to the presence of sensory defects at high intensity may produce some competition phenomena between volatiles in SPME sampling. These phenomena can be reflected in a significant lack of linearity as a consequence of a major difficulty of the adsorbent to extract some volatile compounds over others that have greater affinity to the fiber. The study of these phenomena was structured in two steps: in the first step, the chromatographic area recorded for each marker was studied in relation to the percentage of IOC reference defects present in the resulting mixture. In the second step, the same study was once again implemented but now analyzing the evolution of the concentration of markers (in mg/kg) versus the percentage of reference defect samples in the mixture. This second study involved the analysis of the behavior of the internal standard, 4-methyl-2-pentanol, in matrices with an aroma profile with different degrees of complexity. This internal standard is commonly used for quantifying VOO volatiles.(10, 24) In this case, the objective was to know whether the internal standard may be also affected by a competition phenomenon as a consequence of a complex aroma and to check whether this phenomenon may have an impact on quantitation.

Trend of the Chromatographic Areas of the Markers with Increased Addition of Reference Defect Samples to EVOOs

The competition phenomenon typically occurs when the analyzed amount exceeds the upper limit of the linear range.(17) Thus, the study of the highly odorant samples resulting

from mixing EVOO and reference defect samples revealed a significant lack of linearity in the registered areas for some volatile compounds. The analysis of the trends for all of the analyzed compounds in terms of chromatographic areas plotted against the percentage of reference defect samples added to EVOOs is presented in Table 3. A trend was considered as upward or downward linear (UL or DL) when the RR analysis relating the chromatographic areas and the percentages of reference standard samples results in a squared regression coefficient (R^2_{adj}) of >0.90 . Table 3 shows that some curves were upward nonlinear (UNL) instead of upward linear (UL). As an example, Figure 1 shows the evolution of the chromatographic area of heptanal when the percentages of reference defect samples (fusty, musty, rancid and, vinegary) that are added to EVOO var. Picual are increased. This compound is a volatile marker of rancid oils (3.76 mg/kg; Table 1), which explains why the chromatographic areas abruptly increase as the percentage of rancid reference sample increases. This compound is found at low concentrations in vinegary oils (0.05 mg/kg),(10) which means that the area of heptanal slightly decreases when the vinegary IOC sensory defect is added. The increase of the area of heptanal is almost linear with the addition of fusty or musty IOC reference defects, but it is nonlinear in the case of the addition of rancid reference sample. This nonlinear trend can be explained by the competition phenomenon considering that this compound produces a linear response in the working range of 0.26–3.00 mg/kg, as was proved in a validation study carried out with refined oils.(10) When the compound is extracted from a complex matrix containing a high number of compounds as is the case of a highly rancid oil, the curve exhibits an apparent saturation with percentage of reference samples $>50\%$ (Figure 1). That percentage corresponds to a concentration of 1.88 mg/kg, which is within the linear working range.(10) Considering the rejection threshold determined by panelists for rancid defect (37.5% of reference defect sample), the response of SPME-GC/FID for heptanal becomes nonlinear with percentages higher than this rejection

threshold when rancid reference sample is added. That means that this compound can be accurately determined in the case of slight rancidity, which is the case when a method with an accurate quantitation is needed to support the panel test.(25) The lack of linearity and the competition phenomena can also be explained in terms of sensitivity loss. The curve of chromatographic area of heptanal plotted against the addition of rancid reference sample (Figure 1) shows changes in sensitivity expressed as slope ($\Delta y/\Delta x$) from 7394 (at 10% of added rancid sample) to 3567 (at 50% of added rancid sample, when the rejection threshold is overtaken). This reduction in the slope means 48% of sensitivity loss.

With regard to other volatile compounds, Table 3 shows that all of the selected volatiles, with the exception of aldehydes octanal, nonanal, and (E)-2-heptenal, showed a nonlinear trend, either upward or downward nonlinear (UNL or DNL) with a $R^2_{adj} < 0.80$, when rancid reference sample was added to EVOO from Hojiblanca and Picual varieties. Once again, these nonlinear trends pointed out the competition phenomena of volatiles to be adsorbed by SPME fiber in a static headspace when amount and concentration of volatiles are high, as is the case of highly rancid oils. The percentages of volatiles with nonlinear trends when EVOO samples were spiked with reference defect samples is, in descending order, 65% for rancid defect, 47% for vinegary defect, 41% for fusty defect, and 17% for musty defect. This order corresponds to the order of the typical total amounts of volatile compounds associated with these sensory defects. Thus, Morales et al.(2) pointed out that the total content of volatile compounds is significantly high for rancid oils compared to the other defects, and it is also remarkable that the total concentration of volatiles for musty oil is very low, even similar to that for EVOO.

One of the demanded applications of volatile analysis in VOO sensory assessment is the detection of sensory defects at low intensity of median of defect, when panelists or consumers do not agree on a unanimous classification of the oil.(6) For this application, at

least the trend of the chromatographic areas should be linear at percentages of reference defect samples below the values of the rejection thresholds determined for the panelists. The linearity at these percentages would guarantee an accurate quantitation of volatile markers of sensory defects before the panelists start perceiving the off-flavors. Table 3 also shows that some volatile compounds present a linear response in the range between 0% of added reference defect samples and the rejection threshold (e.g., 0–37.5% in the case of rancidity for heptanoic acid). The nonlinear trend of the other compounds can be corrected by the internal standard, which may be also affected by competition phenomena and therefore may compensate their impact on quantitation. For that reason, a similar study was carried out to evaluate the linearity of the determined concentrations.

Trend of the Concentrations of the Markers with Increased Addition of Reference Defect Samples to EVOOs

Information about the volatiles present in VOOs is not generally given in areas but in concentration by the addition of an IS to the sample, which is 4-methyl-2-pentanol. Thus, the observed trend of the IS should elucidate why a nonlinear evolution of the areas of some volatiles is described as linear when information is given in concentrations (mg/kg).(10)

Ideally, an IS is used to measure or represent loss of target analytes during the sample extraction, normalizing or correcting the errors or variations that happen during the analysis. If the IS fails to account for these analyte losses and variations, then the quantitation will be inaccurate and imprecise.(26) On the other hand, in an ideal case in which the errors are minimum, the determined chromatographic areas for the IS are expected to be constant in all of the samples, because the same amount is added (2.6 mg/kg). Figure 2 shows the trend of the areas of the IS (4-methyl-2-pentanol) when increased percentages of reference defect samples are added to EVOOs var. Hojiblanca and var. Picual. This figure points out that the

chromatographic area is not constant and its variability depends on the percentage of reference defect sample that is added. Furthermore, the influence of the added reference defect samples is more significant in the case of rancid and vinegary defects. The abrupt changes in the area of the internal standard are explained by the competition phenomena between the volatiles present in the gas phase during the adsorption to the SPME fiber in static headspace sampling. Thus, the relative standard deviations (RSD) of the areas of the IS when EVOOs are spiked with increasing percentages of musty and fusty IOC reference defects were 15 and 10%, respectively. By contrast, these values reached 118 and 83% when EVOOs were spiked with rancid and vinegary IOC reference defect, which pointed out the major effect of the competition phenomena when samples with these defects are analyzed.

In this study we can distinguish again three types of trends in the quantitation of volatiles: upward linear (UL), upward nonlinear (UNL), and downward nonlinear (DNL). Figure 3 shows that ethyl acetate, which is a marker of vinegary sensory defect (Table 1), exhibit a chromatographic area that follows a nonlinear trend (UNL; $R^2_{adj} = 0.67$) with the addition of increasing percentages of vinegary reference oil to EVOOs. However, the figure also shows how the IS exerted a change to a linear trend (UL; $R^2_{adj} = 0.98$) when information was given in concentration (mg/kg), whichever the EVOO spiked with reference defect sample. Thus, the IS compensates the competition phenomena between compounds and allows a linear quantitation of this compound.

For the rest of the compounds, Table 3 shows likewise how nonlinear trends of some chromatographic areas become linear ($R^2_{adj} > 0.90$) when the information is given as concentrations. The effect of the IS, the chromatographic areas of which may be affected by the competition phenomena (overall in rancid and vinegary defects), allows determining with a linear response most of the compounds except 12, 24, 35, and 18% of them in the case of rancid, musty, fusty, and vinegary samples, respectively (Table 3). Thus, in rancid oils, where

the competition phenomena were more remarkable, 10 of 15 compounds showing a nonlinear trend in chromatographic areas were determined quantitatively with a linear response due to the compensating effect of the IS. Only 2-heptanol and acetic acid showed no correction when their areas were normalized by the IS (Table 3). However, considering the volatiles that are markers for each defect (Table 1), all of the volatiles exhibit a linear response in the defects where they serve as markers. Thus, for example, although the quantitative determination of acetic acid may be subjected to competition phenomena in the case of highly rancid oils, this compound is a marker of vinegary defect, and it shows a linear response in vinegary mixtures, therefore validating its use as a marker (Table 3).

In conclusion, the competition phenomena between volatile compounds in their adsorption to the SPME fiber are observable when highly odorant oils are being analyzed. These phenomena affect the volatile concentration determination. The particular case of oils with a high intensity of defects has been considered in this study. The competition phenomena have been observed in the analysis of volatiles of these samples in the form of a significant loss of linearity in some compounds, which were not observed in samples with a normal content of volatiles (approximately <100 mg/kg of total volatiles).(10) Thus, a nonlinearity trend was observed in 10 of 17 compounds in the comparison of the chromatographic areas and the percentage of added reference defect samples in at least one of the studied defects. However, the response of SPME-GC/FID analysis of those compounds remained linear in the range of percentages of added reference defect samples below the rejection threshold determined with panelists. It is in those percentages when an accurate determination of volatile markers is needed to certify the presence of defects that are still not perceived by all of the panelists. On the other hand, the study focused on the IS revealed that the effect of the competition phenomena on the chromatographic areas is corrected by the normalization with the IS for all of the volatile compounds in the case of the defects

representative as markers (e.g., acetic acid for vinegary oil) (Table 1). This result validated the utility of these compounds as markers and pointed out that the proposal of any new marker should take into account how it behaves in the case of determination in highly odorant matrices.

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Table 1. Volatiles that are markers of IOC standard oils (rancid, musty, fusty, and vinegary) qualified by their average concentrations, odor thresholds, and sensory characterization.

Peak Number ^a	Volatile	Concentration ^b	Odor Threshold	Sensory Characterization
<i>Rancid</i>				
3	Pentanal	2.63	0.24	Oily, wood, bitter, almond
4	Hexanal	33.80	0.08	Oily, fatty, green, green apple, lawn
5	Heptanal	3.76	0.50	Greasy, rancid
6	Octanal	8.44	0.32	Greasy, soap, fat
8	(E)-2-Heptenal	1.18	4.2×10 ⁻³	Soap, greasy, almond, pungent
10	Nonanal	7.12	0.15	Rancid, fatty, waxes, pungent
14	Butanoic acid	11.50	0.65	Fusty, cheese, strong
16	Hexanoic acid	6.47	0.70	Rancid, sour, sharp
17	Heptanoic acid	0.22	0.10	Rancid, fatty
<i>Musty-humidity</i>				
7	1-Octen-3-one	0.13	0.01	Mushroom, mould, pungent
11	1-Octen-3-ol	0.25	1.0×10 ⁻³	Mould, earthy
<i>Fusty-Muddy Sediment</i>				
1	Octane	2.05	0.94	Alkane, sweet, solvent
9	2-Heptanol	0.13	0.01	Earthy, sweet
13	Propanoic acid	15.60	0.72	Sour, pungent
15	Pentanoic acid	2.48	0.60	Putrid, pungent
<i>vinegary</i>				
2	Ethyl acetate	3.53	0.94	Sticky, sweet, aromatic
12	Acetic acid	6.21	0.50	Sour, vinegary

Note: ^a Referred to elution order of the peaks; ^b determined by DHS-GC (Morales et al., 2005)

Table 2. Rejection thresholds^a expressed in percentage of added reference defect sample to extra virgin olive oils (EVOO) for each one of the sensory defects.

EVOO	Rancid	Fusty	Musty	Vinegary
var. Hojiblanca	17.5	17.5	75	37.5
var. Picual	37.5	37.5	37.5	17.5

Note: ^a, percentages of each reference defect samples in the mixtures with the two EVOOs var. Hojiblanca and Picual at which the 60% of the consumers rejected the sample.

Table 3. Behavior of the markers in the experiments with increasing percentages of IOC reference defects added to EVOOs *var.* Hojiblanca and *var.* Picual^a.

Peak number ^b	Volatile	Rancid		Musty		Fusty		Vinegary	
		Area	Conc ^c	Area	Conc ^c	Area	Conc ^c	Area	Conc ^c
1	Octane	UNL	UL	UL	UL	UL	UL	DNL	DNL
2	Ethyl acetate	DNL	DL	DL ^d	DL ^d	DL ^c	DL ^d	UNL	UL
3	Pentanal	UNL	UL	UL	UL	UNL(H) ^c , UL(P)		DNL	DNL
4	Hexanal	UNL	UL	UL	UL	UL	UL	UNL	UL
5	Heptanal	UNL	UL	UL	UL	UL	UL	UNL	UNL ^d
6	Octanal	UL	UL			UL	UL	UL	UL
7	1-Octen-3-one			UL	UL				
8	(E)-2-Heptenal	UL	UL	DNL	IE	DNL	DNL		
9	2-Heptanol	UNL	UNL(H), UL(P)			UL	UL		
10	Nonanal	UL	UL	UL	UL	UL	UL		
11	1-Octen-3-ol			UL	UL				
12	Acetic acid	UNL	UNL(H), UL(P)			UNL	UNL	UNL	UL
13	Propanoic acid	UNL	UL	UL	UL	UL	UL	UNL ^d	UL
14	Butanoic acid	UNL	UL	UNL ^d	UL(H), UNL(P) ^d	UNL	UL(H), UNL(P)	UNL ^d	UL
15	Pentanoic acid	UNL	UL	UNL ^d	UNL ^d	UNL ^c	UNL ^d	UL	UL
16	Hexanoic acid	UNL	UL			UNL	UL(H), UNL(P) ^d	UL	UL
17	Heptanoic acid	UNL ^c	UL			UNL	UNL	UL	UL

Note: ^a Information from chromatographic areas and concentrations (mg/kg). ^b Referred to elution order of the peaks; ^c, Concentration (mg/kg); (H): *var.* Hojiblanca; (P): *var.* Picual; DL: downward linear; DNL: downward nonlinear; UNL: upward nonlinear; UL: upward linear; ^d, linear behavior in the range between 0% and the rejection threshold by assessors.

FIGURE 1

Figure 1. Trend of the area of heptanal (arbitrary units, a.u.), a marker of rancid sensory perception (Table 1), when adding increasing percentages of reference defect samples (rancid, musty, fusty, vinegary) to EVOO var. Picual. The rejection thresholds are indicated with solid symbols.

FIGURE 2

Figure 2. Trend of the areas of the internal standard when increasing percentages of IOC reference defects are added to EVOOs var. Hojiblanca and var. Picual.

FIGURE 3

Figure 3. Trend of the chromatographic areas and concentrations of ethyl acetate, a marker of vinegary sensory defect, when increasing percentages of vinegary reference sample that is added to extra virgin olive oils *var.* Hojiblanca and *var.* Picual.

TOC FIGURE