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# Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils☆

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**Abstract**

Solid-phase microextraction was used as a technique for headspace sampling of extra virgin olive oil and virgin olive oil samples with different off-flavours. A 100 μm coated polydimethylsiloxane fiber was used to extract volatile aldehydes, the sampling temperature was 45 °C and the fiber has been exposed to the headspace for 15 min. Nonanal and 2-decenal were present in all the olive oils with extraction off-flavours but were not in extra virgin olive oil sample.


*Keywords:* Olive oil; Headspace analysis; Food analysis; Volatile organic compounds

## 1. Introduction

Virgin olive oil is extracted from *Olea europaea* L. fruits using physical methods. Depending on the fruit stage and the extraction process the oil presents different chemical and sensorial characteristics, and it may be classified indifferent quality categories, as established by European Union (EU) Regulations [1]. One of the most important parameter used to oil quality classification is the sensorial analysis. Extra virgin olive oil and virgin olive oil can be consumed directly and the must not shown any off-flavours. Lampante virgin olive oil corresponds to oils that present unacceptable off-flavours and thus, they have to be refined for human consume.

Oxidation is the most frequently occurring form of lipid deterioration, which leads to the development of rancidity, off-flavour compounds causing reduction of shel-life and nutritional value.

The evaluation of oxidation level of an oil can be done using several compounds from the decomposition of hydroperoxide from the main fatty acids: oleic, linoleic and linolenic. 2-Pentenal, hexanal, 2-heptenal, 2-octenal, octanal and nonanal have been related with the rancidity of fats [2–4] being detected in olive oils [5–7]; thus, the ration hexanal/nonanal has been used for detection of oxidised olive oils [8]; 2-octenal were detected in lampante olive oils [9]; hexanal, octanal and 2,4-decadienal have been related with rancidity in food [10–12]. Other oxidation compounds present in olive oil, as 2-hexenal, can be originated from lipoxygenase pathway [13].

Usually these compounds are determined by GC, using different techniques as direct injection, static headspace and dynamic headspace, being the last one is the most used and it has contributed mainly to the knowledge of the aroma composition of virgin olive oil [2,14].

Solid-phase microextraction (SPME) has been introduced as an alternative to the dynamic headspace as preconcentration method previous to GC analysis. This is a faster method, solvents are not required, developed by Artur and Pawliszyn [15] for water analyses and then applied to food analysis.

Recently SPME has been applied for volatile analysis from edible oils stored at high temperatures [12]; has been used to characterise virgin olive oils from Italian regions [16] and to analyse volatiles produced by olive leaves and fruits [4]. Vicchi et al. [9] have evaluated the efficiency of SPME for identification and quantification of volatiles of olive oils.

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The aim of this work is to study the viability of SPME method for defective olive oils detection using a limited compound number related with rancidity of olive oil: 2-hexenal, 2-octenal, nonanal and 2,4-decadienal. The volatiles used in this work have been evaluated as potential markers of defective oils using virgin olive oils with different off-flavours, characterised by panel test.

The fiber polydimethylsiloxane (PDMS) of 100 um has been used, it shows a low sensibility to volatiles but achieve it saturation for these compounds in shorter times (10–15 min) [9] and has a similar level of reproducibility, which is important to reduce the analysis time for its application to real time virgin olive oil characterisation in the oil mill.

## 2. Material and methods

### 2.1. Virgin olive oil samples

The oil samples have been extracted in the industrial oil mill of the CIFA, located in Mengibar (Jaén), from olive fruits of 'Picual' cultivar. The fruits presented different characteristics: picked from the tree, harvested from the ground, long storage and frozen fruits, they were manufactured under different conditions in order to obtain virgin olive oils of extra quality and with different negative attributes: rancid, muddy, fusty, wine-vinegar, waste water and frozen fruit. For this preliminary work seven olive oil samples were used, one for each negative attribute and an extra virgin olive oil.

### 2.2. Standard and reagents

Standards: hexanal, 2-hexenal, 2-heptenal, octanal, 2-octenal, nonanal, 2-decenal and 2,4-decadienal have been supplied by Sigma–Aldrich (St. Louis, MO, USA). A standard test mixture was prepared in a 6 ml vial sealed, 10 μl of each standard was added to 3 g of refined olive oil. A methyl oleate standard was supplied by Sigma–Aldrich.

### 2.3. SPME fiber

A SPME fiber coated with 100 μm of PDMS phase (Supelco, Bellefonte, PA, USA) was used. Previously to analysis, was conditioned by introducing the fiber into injector of gas chromatography system set at 260 °C for 2 h in a stream of Nitrogen.

### 2.4. Temperature and time SPME extraction

To determine the effects of heating temperature and time on the equilibrium of volatile compounds between the SPME coating and headspace, the sample vials were maintained at 30 or 40 °C for 0, 5, 10, 15, 20, 25, 30 and 45 min. These temperatures are similar to those used on dynamic headspace system [5]. At higher temperatures oil oxidation could increase the volatile concentration and undesirable volatile compounds can be arise [2].

To increase the diffusion of flavour compounds, the sample was agitated using a magnetic stirring bar.

### 2.5. Sensorial evaluation

Sensorial analysis was performed by panel test procedure according to EU Regulation 2568/91. The oil samples were evaluated by the analytical Panel Test of Estacion de Olivicultura y Elaiotecnia, constituted by 10 trained and selected panelists. Panelists qualified the samples by flavour descriptors, in a profile sheet, and then a final score (OP), on a 9-point scale, was given. The profile sheet of the EU regulation is divided into two types of sensory attributes, 'positive' and 'negative'. Fusty, muddy, rancid, wine-vinegar, waster water and frost fruit they are negative attributes that indicate defective oils. Final score $\geq$5.5 on the scale, indicates a better olive oil quality; score $\leq$3.5 indicates a poor olive oil quality.

### 2.6. Gas chromatography

Volatile analyses have been performed using a HP 6850 serie II gas chromatograph equipped with a flame ionization detection (FID) system and a splitt/splittless injector. For chromatographic separation a HP5 column 30 m long, internal diameter 0.25 mm and a 0.25 μm film thickness (Agilent Technologies, USA) was used. The initial oven temperature was maintained at 40 °C for 5 min, and then increased at 4 °C $min^{-1}$ up to 220 °C, the final temperature was held for 10 min. The injector temperature was 260 °C and the detector 300 °C. The carrier gas was nitrogen at a pressure of 15 p.s.i. (1 p.s.i. = 6894.76 Pa) at the column head.

### 2.7. Microextraction process and desorption

A 3 ± 0.01 g virgin olive oil sample was weighted into a 6 ml vial containing a microstirring bar and sealed. The SPME fiber was inserted into the headspace of the vial, the volatiles were then adsorbed on the fiber. The fiber was introduced into the injection port equipped with a 0.75 mm i.d. glass liner (Supelco) operated at 260 °C in splittless mode and kept for 3 min for the desorption of flavour compounds.

### 2.8. Olive oil oxidation level

The quality parameter K270 was performed according to EU regulation 2568/91.

### 2.9. Qualitative and quantitative analysis

Compounds were identified by comparison of their retention time with those standard compounds. Relative percentage, as percent normalized areas, was used for quantification.

## 3. Results and discussion

### 3.1. Temperature and time SPME extraction

The effect of heating temperature and extraction time on volatile aldehydes: 2-hexenal, 2-octenal, nonanal and 2,4-decadienal are shown in Fig. 1. The GC peak area of each compound increased as the heating temperature from 30 to 45 °C. For both temperatures, 2-hexenal and 2-octenal reached the equilibrium at 5 min; nonanal reaches equilibrium for 15 min at 30 °C and 5 min at 45 °C, whereas 2,4-decadienal needed longer time when the extraction was performed at 30 °C and 30 min at 45 °C.

For this work the optimal SPME sampling conditions were 45 °C for 15 min, obtaining a good peak area reproducibility for 100 μm of PDMS fiber. The coefficients of variation for the standards, from five replicates, ranged between 4.9% for nonanal and 10.9% for 2,4-decadienal.

### 3.2. Olive oil sample analysis

Sensorial characterisation and oxidation level for oil samples are showed in Table 1. Sample 1 was an extra virgin olive oil, without off-flavours and lower oxidation level. Samples 2 and 3 are ordinary virgin olive oils with frozen fruit and muddy sediment off-flavours, respectively. The rest of samples, 4–7, were lampante virgin oils with wine-vinegar, fusty, waste waters and rancid off-flavours, respectively, and high oxidation level.

Table 1
Sensorial characterization and oxidation level of virgin olive oil samples

| Olive oil samples | Final score (OP)[a] | Principal negative atributes[b] | K270 |
|---|---|---|---|
| 1 | 7.5 | Not detected off-flavours | 0.11 |
| 2 | 5.0 | Frost fruit, rancid | 0.16 |
| 3 | 4.5 | Muddy sediments, mushroom | 0.19 |
| 4 | 3.0 | Wine vinegar, rancid | 0.25 |
| 5 | 3.0 | Fusty, rancid | 0.31 |
| 6 | 2.0 | Waste water, rancid | 0.56 |
| 7 | 2.0 | Rancid | 0.56 |

[a] For a 0–9 point scale.
[b] For a 0–5 point scale. Only ≥3 are shown.

The presence of volatile aldehydes in virgin olive oil samples has been analysed by SPME–GC–FID. Compound identification was established comparing the retention times with those of the standards from test mixture. In Table 2, retention times ($t_r$) and the relative percentage, as normalized peak area, of the aldehydes extracted from the different olive oil samples are indicated.

As can be observed, from the proposed volatiles only hexanal and 2-hexenal were found in extra virgin olive oil while the rest of volatiles have not been detected.

From the SPME–GC–FID data can be observed as for defective virgin olive oils nonanal was found at percentages ranged between 0.56% for sample 4 (wine vinegar defect)

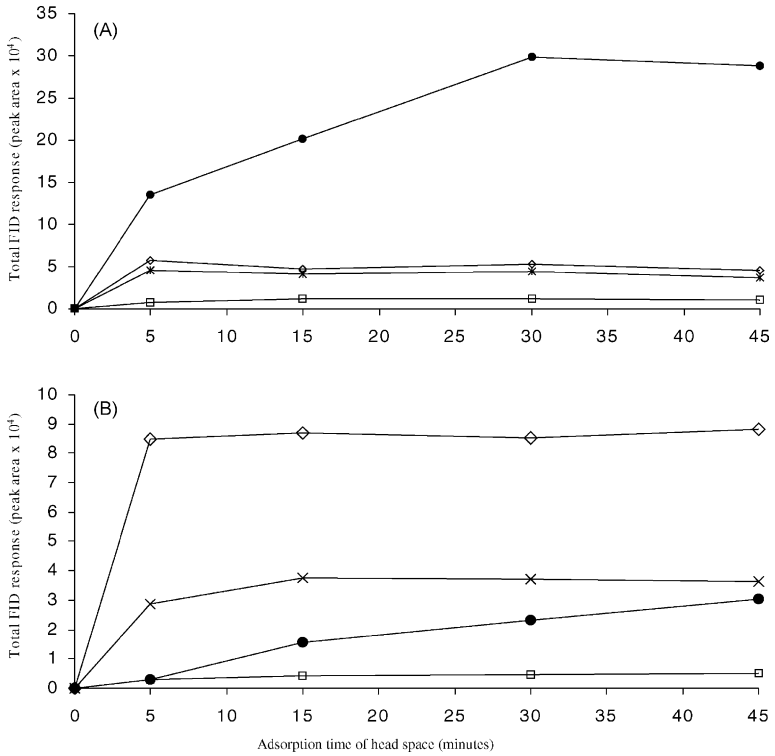


Fig. 1. Effects of temperature and time on the equilibrium of volatile aldehydes between the SPME coating, using a 100 μm polydimethylsilosane (PDMS) fiber, and the headspace of standards mixture test. (A) Heating temperature of 45 °C. (B) Heating temperature of 30 °C. (◇) 2-Hexenal; (×) 2-octenal; (□) nonanal; (●) 2,4-decadienal.

Table 2
Relative percentage (percent normalised areas) of the volatile aldehydes extracted by SPME from virgin olive oil samples, calculated from chromatographic data

| Compound | $t_r$ (min)[a] | Olive oil samples | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| (1) Hexanal | 3.05 | 13.58 | 20.81 | 9.27 | 0.45 | 6.92 | 24.73 | 22.05 |
| (2) (*E*)-2-Hexenal | 4.44 | 6.88 | – | – | – | – | – | – |
| (3) (*E*)-2-Heptenal | 8.61 | – | 7.04 | – | 1.15 | – | – | 0.59 |
| (4) Octanal | 10.63 | – | 3.54 | – | 0.12 | – | 3.17 | 6.88 |
| (5) (*E*)-2-Octenal | 12.98 | – | – | – | – | – | 1.05 | |
| (6) Nonanal | 14.95 | – | 8.58 | 9.49 | 0.56 | 6.68 | 6.4 | 9.8 |
| (7) (*E*)-2-Decenal | 20.90 | – | 5.24 | 7.26 | 0.37 | 4.56 | 5.72 | 6.57 |
| (8) 2,4-Decadienal | 22.95 | – | – | – | – | – | 2.18 | – |
| Total peak areas ($\times 10^4$) | | 3.352 | 4.836 | 4.252 | 53.85 | 5.31 | 13.07 | 37.81 |

[a] Retention times from standard test mixture.

and 9.80% for sample 7 (described as rancid). 2-Octenal and 2,4-decadienal appeared in virgin olive oils with waste water off-flavour. 2-Hexenal was detected only in extra virgin olive oil sample. Other aldehyde volatile compounds detected are: hexanal, that has been found in the whole of samples at percentages ranged between 0.45% for wine vinegar defect and 24.73% for waste water defect; 2-heptenal in oils with frozen fruit, wine vinegar and racind defects; octanal in waste water, frozen fruit, wine vinegar and rancid defects. Finally, from de chromatographic data, the presence of a compound with retention times, $t_r = 20.90$ min, only in defective oils, can be observed. In assays of thermoxidation of methyl oleate appears a peak with the same retention indexes. This volatile compound has been associated with 2-decenal, formed from decomposition of 9-hydroperoxy-10-ene of oleic acid [17].

To evaluate the oxidation level of oil samples the parameter K270 has been used, data are shown in Table 1. EU regulation establishes a value of K270 $\geq$ 0.25 for lampante virgin olive oil (poor quality). Samples 1, 2 and 3 showed values lower than 0.25, however the sensorial score (OP) lot classify sample 1 as extra virgin olive oil while samples 2 and 3 showed off-flavours and thus classified as deffectives. Nonanal and 2-decenal are present on chromatographic data from 2 and 3 samples, at higher percentage. A higher percentage for these aldehydes volatiles and higher K270 value, 0.56, are found in sample 7, rancid is the principal off-flavour for this. Vichi et al. [9] indicates a relation on peroxide value which unsaturated aldehydes and higher level on nonanal are founded in olive oil samples which a higher peroxide.

In conclusion, application of SPME extraction to virgin olive oil flavour with a 100 μm PDMS fiber, for the heating temperature and time on the equilibrium condition found, provide information about olive oil defectives through the volatile aldehydes extracted. Only nonanal and 2-decenal can provide discrimination between extra virgin olive oil and defective virgin olive oils.

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