



METHOD OF ANALYSIS

FOR THE DETERMINATION OF VOLATILE COMPOUNDS IN VIRGIN OLIVE OIL BY SPME-GC-FID/MS

SCOPE

This method has been developed for the determination of the content of selected volatile compounds in the headspace of virgin olive oils. The volatile compounds are responsible for the aroma of virgin olive oils, and in particular for positive attributes or sensory defects. The selected volatile compounds represent the minimum number of diagnostic molecules responsible for fermentative and non-fermentative defects. Thus, fusty-muddy sediment defect is associated to octane, ethanol, 3-methyl-1-butanol, propanoic acid, 6-methyl-5-hepten-2-one, winey-vinegary defect to acetic acid, ethyl acetate, ethanol and musty-humid-earthly to (*E*)-2-heptenal, 1-octen-3-ol, propanoic acid), frostbitten olives defect to ethyl propanoate, and rancid defect to hexanal, nonanal, (*E,E*)-2,4-hexadienal, (*E*)-2-decenal, pentanoic acid. In addition, three compounds [(*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, 1-hexanol] were included given their relationship with fruity attribute.

PRINCIPLE

This method is based on the isolation and preconcentration of volatile compounds present in the headspace of virgin olive oils and responsible for aroma by solid phase microextraction (SPME) and subsequent separation of the analytes by gas chromatography (GC) and quantification with a flame ionization detector (FID) or mass spectrometry (MS).

DIFFERENCES

Both methods allow the quantification of volatile compounds by SPME-GC with two possible detectors, and the use of one detector or the other depends on the technical facilities of the laboratories applying this method; the double choice is designed to favor maximum application of the method.

1. APPARATUS

- 1.1. Glass vial, 20 mL.
- 1.2. Septum and aluminium seals or seals adaptable to the autosampler if the latter is used.
- 1.3. Gas chromatograph (GC), suitable for use with a capillary column, equipped with a split/splitless injector and a mass spectrometry detector (MS) / flame ionization detector (FID). Depending on the detector available in the laboratory, the gas chromatographic analysis can be carried out with one of the two detectors (see section 3.2 “Gas chromatography analysis (choose one of two options)” below).
- 1.4. SPME injector liner, 0.75 mm ID.
- 1.5. Capillary column, fused silica, a polar phase based on polyethylene glycol (PEG) (e.g. ZB-WAX or TR-WAX), length 60 m, internal diameter 0.25 mm, and coating 0.25 – 0.50 μm .
- 1.6. SPME fiber, length 1 cm, 50/30 μm film thickness, and it is endowed with the Stable Flex stationary phase of divinylbenzene/carboxen/polydimethylsiloxane. The fiber would be previously conditioned by following the instructions of the supplier.
- 1.7. Analytical balance for weighing to an accuracy of ± 0.1 mg.

2. REAGENTS

- 2.1. 4-methyl-2-pentanol, chromatography grade, with a purity higher than 95%.
- 2.2. Analytical standards

Octane: CAS Number 111-65-9; assay $\geq 99.7\%$; density 0.703 g/mL at 25°C;

Ethanol: CAS Number 64-17-5; assay $\geq 99.9\%$; density 0.7893-0.7899 g/mL at 20°C;

3-Methyl-1-butanol: CAS Number 123-51-3; assay $\geq 98.5\%$; density 0.809 g/mL at 25°C;

Propanoic acid: CAS Number 79-09-4; assay $\geq 99.8\%$; density 0.993 g/mL at 25°C;

6-methyl-5-hepten-2-one: CAS Number 110-93-0; assay $\geq 97.0\%$; density 0.855 g/mL at 25°C;

Acetic acid: CAS Number 64-19-7; assay $\geq 99.8\%$; density 1.049 g/mL at 25°C;

Ethyl acetate: CAS Number 141-78-6; assay $\geq 99.8\%$; density 0.902 g/mL at 25°C;

(*E*)-2-heptenal: CAS Number 18829-55-5; assay $\geq 95\%$; density 0.857 g/mL at 25°C;

1-octen-3-ol: CAS Number 3391-86-4; assay $\geq 98.0\%$; density 0.83 g/mL at 25°C;

Ethyl propanoate: CAS Number 105-37-3; assay $\geq 99.7\%$; density 0.888 g/mL at 25°C;

Hexanal: CAS Number 66-25-1; assay 98 %; density 0.834 g/mL at 25°C;

Nonanal: CAS Number 124-19-6; assay $\geq 95\%$; density 0.827 g/mL at 25°C;

(*E,E*)-2,4-hexadienal: CAS Number 142-83-6; assay $\geq 95.0\%$; density 0.871 g/mL at 25°C;

(*E*)-2-decenal: CAS Number 3913-81-3; assay $\geq 95.0\%$; density 0.841 g/mL at 25°C;

Pentanoic acid: CAS Number 109-52-4; assay $\geq 99.8\%$; density 0.939 g/mL at 25°C;

(*E*)-2-hexenal: CAS Number 6728-26-3; assay $\geq 97.0\%$; density 0.846 g/mL at 25°C;

(*Z*)-3-hexenyl acetate: CAS Number 3681-71-8; assay $\geq 98.0\%$; density 0.897 g/mL at 25°C;

1-hexanol: CAS Number 111-27-3; assay $\geq 99.9\%$; density 0.814 g/mL at 25°C.

- 2.3. n-Alkanes, from 8 to 20 carbon atoms, custom blend standard (~40 mg/L each, in hexane).
- 2.4. Carrier gas: helium (or hydrogen if the equipment and installation are adapted and allow for safety procedure; attention should be paid to eventual formation of interferences), pure, gas chromatography grade.
- 2.5. Auxiliary gases: in the case of using nitrogen for conditioning/cleaning the fiber with an autosampler, use gas chromatography grade.

3. PROCEDURE

Before starting the analysis of the samples, build the external calibration curves following the protocol below. The integration method for obtaining the chromatographic areas should be the same in both the calibration curves and the analytes in the sample.

3.1. PROTOCOL FOR THE PREPARATION OF CALIBRATION CURVES FOR VOLATILE ANALYSIS (SPME-GC-MS AND SPME-GC-FID)

The calibration curves are prepared by using two standard mixtures (SM)*, named SM A and SM B, each containing different analytes as described below (Table 1):

Table 1. Standards of volatile compounds included in the two standard mixtures.

Low concentration mixture (SM A) (0.05-10.00 mg/kg)	High concentration mixture (SM B) (0.20-25.00 mg/kg)
Octane	Ethanol
Ethyl acetate	Hexanal
Ethyl propanoate	(<i>E</i>)-2-Hexenal
3-Methyl-1-butanol	(<i>Z</i>)-3-Hexenyl acetate
(<i>E</i>)-2-Heptenal	1-Hexanol
6-Methyl-5-hepten-2-one	Nonanal
(<i>E,E</i>)-2,4-Hexadienal	1-Octen-3-ol
Propanoic acid	Acetic acid
(<i>E</i>)-2-Decenal	
Pentanoic acid	

*In order to minimize competition phenomena between volatile compounds, the standards are divided into two mixtures (SM A and SM B).

Note: SM A and SM B must be stored at -18°C and, for their subsequent use, some precautions must be followed: leave the two mixtures for an adequate time at room temperature (never heating), shake carefully before use, and return them to the freezer. Moreover, it is very important to never leave the vials open and never keep them at room temperature for an extended period of time. Work at controlled room temperature (T = 20-25°C) due to the volatility of the standards. A rapid preparation of the solutions is advisable to avoid evaporation of compounds.

Note: for calculation of the concentrations, use the exact concentration of each standard in SM A and SM B.

3.1.1. Preparation of Standard Mixtures (SM) at 10000 mg/kg

For the low concentration mixture (SM A):

1. Put an empty vial of 20 mL in the analytical balance and tare it.
2. Weigh 5.000 ± 0.001 g of refined olive oil in the vial.
3. Put approx. 0.100 ± 0.001 g for each standard, exactly weighed, for the mixture A (low concentration, 10 compounds) in the vial.
4. Add refined olive oil up to reach approx. 10.000 ± 0.001 g, exactly weighed.
5. Close the vial (cap + septum).
6. Shake for 30 seconds on the agitator (e.g. vortex).

Note: do not forget to write down the weights for concentration calculation.

For the high concentration mixture (SM B):

1. Put an empty vial of 20 mL in the analytical balance and tare it.
2. Weigh 5.000 ± 0.001 g of refined olive oil in the vial.
3. Put approx. 0.100 ± 0.001 g for each standard, exactly weighed, for mixture B (high concentration, 8 compounds) in the vial.
4. Add refined olive oil up to reach approx. 10.000 ± 0.001 g, exactly weighed.
5. Close the vial (cap + septum).
6. Shake for 30 seconds on the agitator (e.g. vortex).

Note: do not forget to write down the weights for the concentration calculation.

3.1.2. Preparation of dilutions from the two SM (A and B)

Note: the following procedure has to be applied in the same way, both from SM A and SM B

SM1 (200 mg/kg):

1. Place a vial (labelled as SM1) of 20 mL on the analytical balance and tare it.
2. Weigh 5.000 ± 0.001 g of refined olive oil.
3. Put approx. 0.200 ± 0.001 g of SM, exactly weighed, in the vial.
4. Add refined olive oil up to reach approx. 10.000 ± 0.001 g, exactly weighed.
5. Close the vial (cap + septum).
6. Shake for 30 seconds on the agitator (e.g. vortex).

Note: do not forget to write down the weights for the concentration calculation.

SM2 (20 mg/kg):

1. Place a vial (labelled as SM2) of 20 mL on the analytical balance and tare it.
2. Weigh 5.000 ± 0.001 g of refined olive oil.
3. Put approx. 1.000 ± 0.001 g of SM1, exactly weighed, in the vial.
4. Add refined olive oil up to reach approx. 10.000 ± 0.001 g, exactly weighed.
5. Close the vial (cap + septum). Shake for 30 seconds on the agitator (e.g. vortex).

Note: do not forget to write down the weights for the concentration calculation.

SM3 (2 mg/kg):

1. Place a vial (labelled as SM3) of 20 mL on the analytical balance and tare it.
2. Weigh 5.000 ± 0.001 g of refined olive oil.
3. Put approx. 1.000 ± 0.001 g of SM2, exactly weighed, in the vial.
4. Add refined olive oil up to reach approx. 10.000 ± 0.001 g, exactly weighed.
5. Close the vial (cap + septum).

6. Shake for 30 seconds on the agitator (e.g. vortex).

Note: do not forget to write down the weights for the concentration calculation.

3.1.3. Preparation of dilutions for building the calibration curves

For the low concentration mixture (SM A), it is necessary to prepare the following 12 dilutions starting from SM1, SM2 or SM3 (see the procedure and Table 2): 0.05, 0.10, 0.15, 0.20, 0.25, 0.5, 1.00, 1.50, 2.00, 2.50, 5.00, 10.00 mg/kg. For the high concentration mixture (SM B), it is necessary to prepare 12 dilutions starting from SM1, SM2 or SM3 (see the procedure and Table 2): 0.20, 0.25, 0.5, 1.00, 1.50, 2.00, 2.50, 5.00, 10.00, 15.00, 20.00, 25.00 mg/kg.

1. Place each vial (20 mL) on the analytical balance and tare it.
2. Weigh 1.000 ± 0.001 g of refined olive oil
3. Add 0.100 ± 0.001 g of IS dilution¹.
4. Add the appropriate weight (Column 5 of Table 2) of SM_x (Column 1 of Table 2) in the vial. The approximate obtained concentration is indicated in the column 6 of Table 2, although the exact concentration needs to be calculated.
5. Add refined olive oil up to reach approx. 2.000 ± 0.001 g, exactly weighted (Column 3 shows the refined olive oil approximately to be added).
6. Close the vial quickly with cap and septum.
7. Shake the vials gently and softly (never spread the oil through the vial walls or the septum).

Note: do not forget to write down the weights for the concentration calculation; ¹, Preparation of IS dilution explained in the section "Standard solution and sample preparation (SPME-GC-MS and SPME-GC-FID)", IS in refined olive oil at a concentration of about 50 mg/kg. Figure 1 shows the chromatograms of SM A and SM B, added with IS and both SM at 20.00 mg/kg, analysed by SPME-GC-FID/MS.

Table 2. Preparation of dilutions for building the calibration curves.

SM _x	Conc. ¹ (mg/kg)	Weight of Refined Oil (g)	Weight of IS dilution ² (g) (2.5 mg/kg)	Weight of SM _x (g)	Final Conc. of volatile (mg/kg)
SM3	2 mg/kg	0.85	0.1	0.05	0.05
		0.80		0.10	0.10
		0.75		0.15	0.15
		0.70		0.20	0.20
		0.65		0.25	0.25
SM2	20 mg/kg	0.85		0.05	0.5
		0.80		0.10	1.00
		0.75		0.15	1.50
		0.70		0.20	2.00
		0.65		0.25	2.50
SM1	200 mg/kg	0.85		0.05	5.00
		0.80		0.10	10.00
		0.75		0.15	15.00
		0.70		0.20	20.00
		0.65		0.25	25.00

Note: ¹, Conc., concentration; ², Preparation of IS dilution explained in the section "Standard solution and sample preparation (SPME-GC-MS and SPME-GC-FID)", IS in refined olive oil at a concentration of approximately 50 mg/kg (final concentration about 2.5 mg/kg).

3.1.4. Sequence of the GC analysis for building the calibration curves

In the sequence of the chromatographic analyses, set the higher concentrations at the end of the sequence, and analyse the blank samples (empty vials) and blank refined oil (oil without compounds added). To avoid interferences in the analysis, only fresh (without evidence of rancidity) refined oils should be used.

The following example is for the low concentration mixture (SM A):

1. Blank (Empty vial)
2. Blank of the matrix (Refined olive oil "2.0 g")
3. Blank of the matrix + IS (Refined olive oil "1.9 g" + IS "0.1 g")
4. Blank (Empty vial)
5. 0.05 mg/kg vial
6. 0.1 mg/kg vial
7. 0.15 mg/kg vial
8. 0.20 mg/kg vial
9. Blank (Empty vial)
10. 0.25 mg/kg vial

11. 0.5 mg/kg vial
12. 1 mg/kg vial
13. 1.5 mg/kg vial
14. Blank (Empty vial)
15. 2 mg/kg vial
16. 2.5 mg/kg vial
17. 5 mg/kg vial
18. 10 mg/kg vial
19. Blank (Empty vial)

Note: one injection at each concentration level is enough for building the calibration curves.

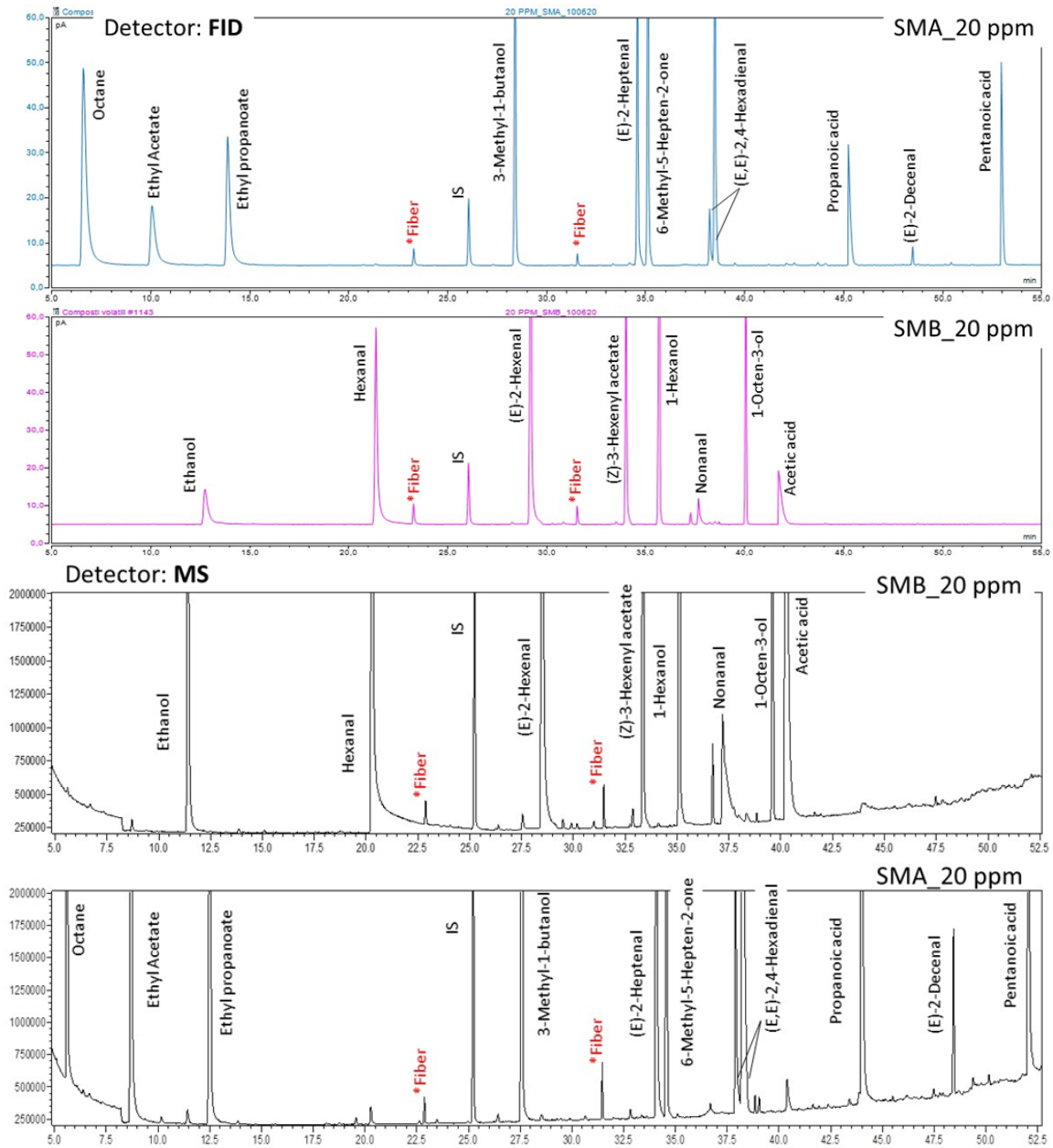


Figure 1. Chromatograms of SM A and SM B, added with IS, at 20 mg/kg (SPME-GC-FID/MS).

*Compounds deriving from the SPME Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber.

3.2. **PROTOCOL FOR THE PREPARATION OF THE STANDARD SOLUTION AND SAMPLE PREPARATION (SPME-GC-MS AND SPME-GC-FID)**

Note: work at controlled room temperature ($T = 20\text{-}25^{\circ}\text{C}$) due to the volatility of the standards. Samples must be at room temperature before sample preparation.

3.2.1. Preparation of the stock standard solution

Weigh 15.000 ± 0.001 g of refined olive oil in a vial, then add 0.100 ± 0.001 g, exactly weighed, of 4-methyl-2-pentanol and add refined olive oil up to reach 20.000 ± 0.001 g, exactly weighed (approximate concentration of 5000 mg/kg). Take note of exact weights (± 0.001 g) for calculation of concentration. A rapid preparation of the stock standard solution is advisable to avoid evaporation of internal standards.

3.2.2. Preparation of standard solution

Weigh 5.000 ± 0.001 g of refined olive oil in a vial, then add 0.100 ± 0.001 g of the stock standard solution of 4-methyl-2-pentanol, exactly weighed, and add refined olive oil up to reach 10.000 ± 0.001 g, exactly weighed (approximate concentration of 50 mg/kg). Take note of exact weights (± 0.001 g) for calculation of concentration. A rapid preparation is advisable to avoid evaporation of internal standards.

3.2.3. Sample preparation

Weight exactly 1.900 ± 0.001 g of virgin olive oil (sample) in a 20 mL glass vial and add 0.100 ± 0.001 g of 4-methyl-2-pentanol standard solution (3.1.2) as internal standard (IS approximate concentration = 2.5 mg/kg). Take note of exact weights (± 0.001 g) for calculation of concentration. Hermetically close the vial with polytetrafluoroethylene septum (PTFE). Shake the vial manually, but very gently and softly (never spread the oil through the vial walls or the septum). Leave the vial at 40°C under agitation (250 rpm in continuous is suggested) for 10 min to allow for the equilibration of the volatiles in the headspace. After the equilibration time, the septum covering each vial is pierced with an SPME needle, and the fiber is exposed to the headspace for 40 min.

3.2.4. Gas chromatography analysis (choose one of two options):

With FID: The volatiles adsorbed by the fiber are thermally desorbed in the hot injection port of a GC for 5 min at 250°C with the purge valve off (splitless mode) and deposited onto a capillary column of a GC with a FID at 260°C . The carrier gas is helium (or hydrogen if the equipment and installation are adapted and allow for safety procedure), at a flow rate of 1.5 mL/min (this is a suggestion, the flow rate must be adjusted to optimize the separation of peaks depending on the use of helium or hydrogen as carrier gas). The oven temperature is held at 40°C for 10 min and then programmed to increase by $3^{\circ}\text{C}/\text{min}$ to a final temperature of 200°C . A cleaning step can be added ($20^{\circ}\text{C}/\text{min}$ to 250°C and hold the temperature for 5 min).

With MS: The volatiles adsorbed by the fiber are thermally desorbed in the hot injection port of a GC for 5 min at 250°C with the purge valve off (splitless mode) and deposited onto a capillary column of a GC with a mass spectrometry (MS) detector. The transfer line temperature is set at 260°C . The temperature of the ion source is set according to the technical specifications of the instrument. The carrier gas is helium (or hydrogen if the

equipment and installation are adapted and allow for safety procedure), at a flow rate of 1.5 mL/min (this is a suggestion, the flow rate must be adjusted to optimize the separation of peaks depending on the use of helium or hydrogen as carrier gas). The oven temperature is held at 40°C for 10 min and then programmed to increase by 3°C/min to a final temperature of 200°C. A cleaning step can be added (20°C/min to 250°C and hold the temperature for 5 min).

3.2.5. Peak identification

The identification of the volatile compounds is carried out by MS (Table 3) if available, checked with standards, and by comparison with the linear retention indexes (LRI).

Table 3. Characteristic relative m/z of the 18 selected compounds used to build the calibration curves.

Code	Compound	Characteristic m/z
1	Octane	85
2	Ethyl acetate	43
3	Ethanol	31, 45
4	Ethyl propanoate	57
5	Hexanal	44
6	3-Methyl-1-butanol	55, 70
7	(<i>E</i>)-2-Hexenal	69, 83
8	(<i>Z</i>)-3-Hexenyl acetate	67, 82
9	(<i>E</i>)-2-Heptenal	83
10	6-Methyl-5-hepten-2-one	108
11	1-Hexanol	56
12	Nonanal	98
13	(<i>E,E</i>)-2,4-Hexadienal	81
14	1-Octen-3-ol	57
15	Acetic acid	60
16	Propanoic acid	74
17	(<i>E</i>)-2-Decenal	70
18	Pentanoic acid	60, 73

3.2.6. Calculation of Linear Retention Index

To identify each extracted compound, the linear retention indexes (LRI) are determined. The mixture of n-alkanes is injected into the GC system; the retention times of the alkanes are used in the following equation, obtaining the LRI of each analyte extracted.

$$\text{LRI} = 100 \times z + 100 \times \left(\frac{(\text{RT}_{\text{analyte}} - \text{RT}_z)}{(\text{RT}_{z+1} - \text{RT}_z)} \right)$$

z is the number of carbon of the alkane that elutes before the molecule, the RT_{analyte} , the RT_z , and the RT_{z+1} are the retention time of the analyte of interest, of the alkane that elutes before and the one that elutes after.

3.2.7. Quantitative analysis

The quantification of selected volatile compounds is carried out by a calibration based on the internal standard and the external calibration curve. The IS is also added in the sample because it is necessary for evaluating the goodness of the fiber sampling and of the whole analytical procedure. Thus, an abrupt change in the peak area assigned to IS, or in their usual RT, would mean a change in the conditions that need to be checked.

External calibration curve ($A_{\text{Analyte}}/A_{\text{I.S.}}$ vs. C_{Analyte}):

$$A_{\text{Analyte}}/A_{\text{I.S.}} = m \cdot C_{\text{Analyte}}, (Y = m \cdot X).$$

$$C_{\text{Analyte}} = (A_{\text{Analyte}} / A_{\text{I.S.}}) / m$$

Where:

A_{Analyte} is the area corresponding to the analyte.

$A_{\text{I.S.}}$ is the area corresponding to the IS used in building the calibration curves.

m , the slope of the calibration curve (built for the selected analyte).

To build the external calibration curves, see “Protocol for the preparation of calibration curves for volatile analysis (SPME-GC-MS and SPME-GC-FID)”.

The integration method for obtaining the chromatographic areas should be the same in both the calibration curves and the analytes in the sample.

Note: Analysis should be carried out in triplicate. The means will be calculated from the areas of those triplicates.

Note: Please, use the provided Excel file to make these calculations.

Note: An abrupt reduction of the IS area can be observed in the case of lampante olive oils with a high median of defect.

4. EXPRESSION OF RESULTS

The content of each volatile compound is expressed in mg/kg, with 3 decimal places.

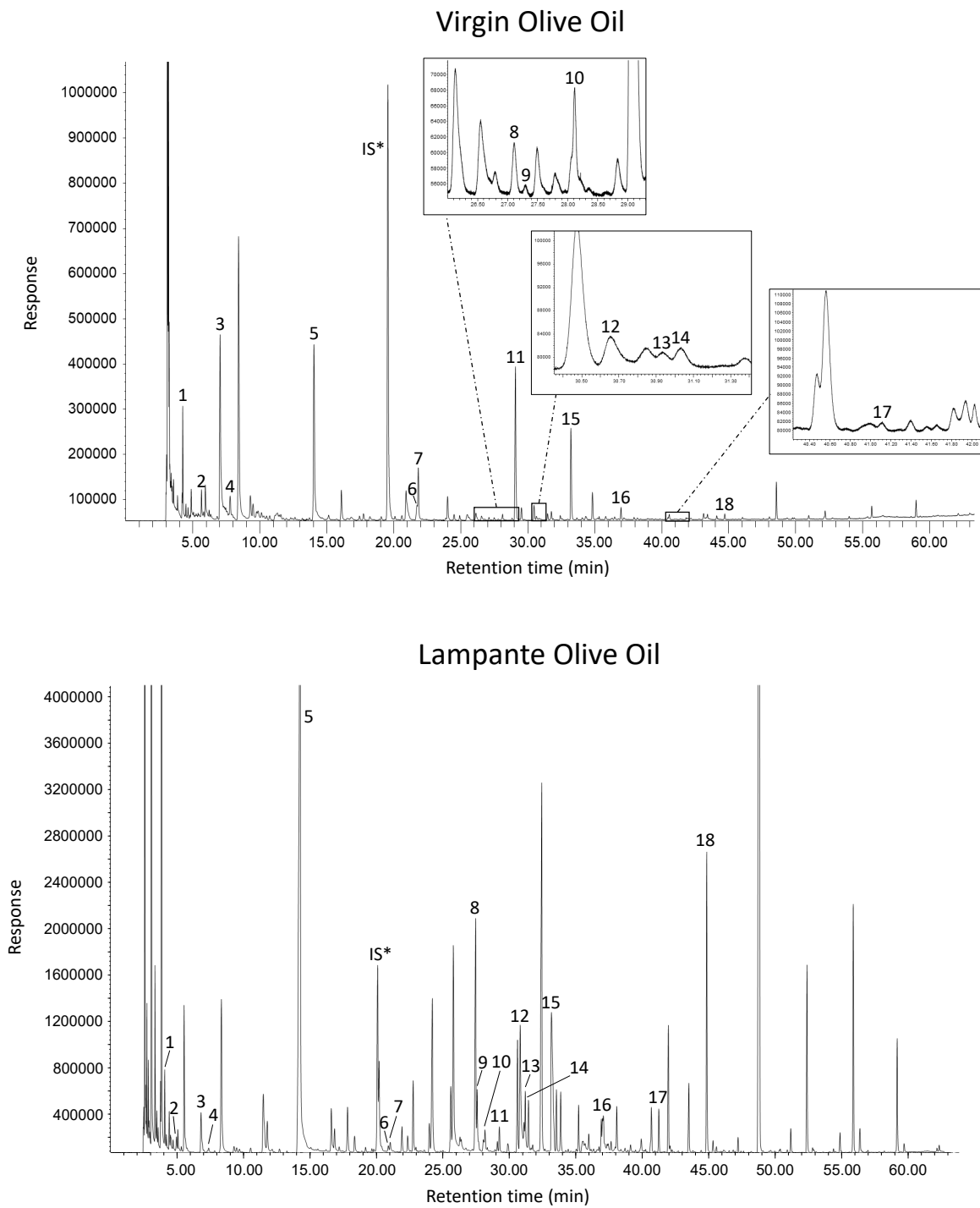
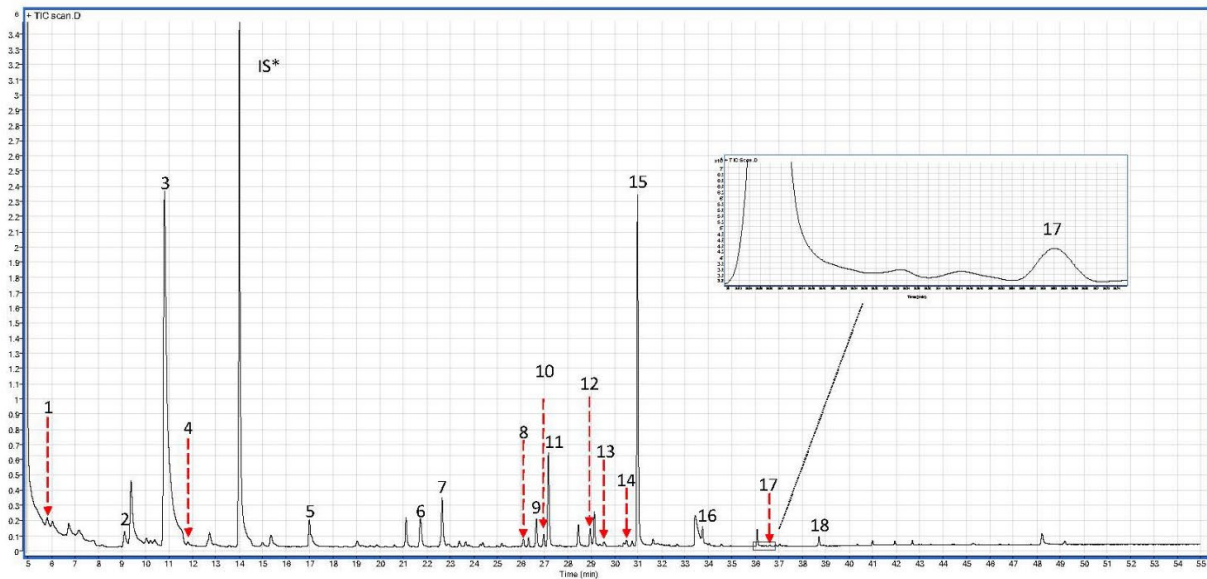


Figure 2. Example of a SPME-GC-FID chromatogram (response, arbitrary units) of virgin volatile compounds identified in a sample of virgin olive oil and lampante olive oil. Numbers refers to the codes specified in Table 3.

Virgin Olive Oil



Lampante Olive Oil

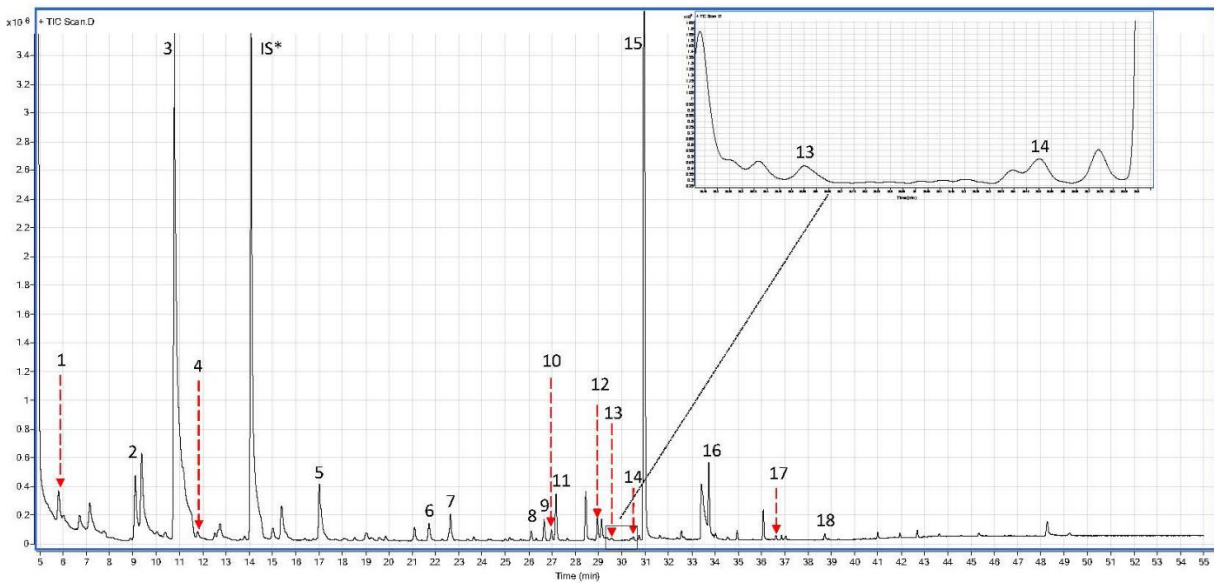


Figure 3. Example of a SPME-GC-MS chromatogram (response, arbitrary units) of virgin volatile compounds identified in a sample of virgin olive oil and lampante olive oil. Numbers refers to the codes specified in Table 3.

PRECISION VALUES OF THE METHOD

Analysis of the collaborative test results for the determination of volatile compounds:

The precision values of the method are given in the table on the next pages for each parameter studied:

n number of laboratories which participated in the test

outliers number of laboratories with outlying values

mean mean of the accepted results

r repeatability

S_r repeatability standard deviation

RSD_r(%) repeatability coefficient of variation ($S_r \times 100 / \text{mean}$)

R reproducibility

S_R reproducibility standard deviation

RSD_R(%) reproducibility coefficient of variation ($S_R \times 100 / \text{mean}$)

The results of the interlaboratory test to determine the volatile compounds are presented for the two possible detectors (FID and MS). An interlaboratory test was carried out in 2019 in accordance with ISO 5725. Following this protocol, the samples described below were provided to the laboratories as paired blind samples. The results are summarised in Tables A.1 to A.36.

A: Virgin olive oil.

B: Extra virgin olive oil.

C: Lampante olive oil.

D: Virgin olive oil.

E: Virgin olive oil.

Table A.1—Octane (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	0	0	0	0	0
Mean (mg/kg)	1.541	0.162	0.244	0.450	1.249
r	0.532	0.054	0.041	0.096	0.237
S_r	0.190	0.019	0.015	0.034	0.084
RSD_r (%)	12.3	11.9	6.0	7.6	6.8
R	0.944	0.139	0.161	0.354	1.210
S_R	0.337	0.050	0.058	0.126	0.432
RSD_R (%)	21.9	30.5	23.6	28.1	34.6

Table A.2—Octane (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	0	1	1	0	1
Mean (mg/kg)	1.552	0.161	0.219	0.377	1.438
r	0.191	0.032	0.050	0.041	0.111
S_r	0.0683	0.0115	0.0180	0.0145	0.0396
RSD_r (%)	4.4	7.2	8.2	3.9	2.8
R	1.391	0.202	0.259	0.437	1.414
S_R	0.4967	0.0721	0.0924	0.1559	0.5051
RSD_R (%)	32.0	44.9	42.2	41.4	35.1

Table A.3—Ethyl acetate (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	1	1	1	1	1
Mean (mg/kg)	2.892	0.542	0.448	0.193	1.035
r	0.536	0.089	0.053	0.053	0.191
S_r	0.191	0.032	0.019	0.019	0.068
RSD_r (%)	6.6	5.9	4.3	9.8	6.6
R	1.004	0.130	0.151	0.129	0.654
S_R	0.359	0.046	0.054	0.046	0.234
RSD_R (%)	12.4	8.5	12.1	23.9	22.6

Table A.4—Ethyl acetate (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	0	1	1	0	1
Mean (mg/kg)	3.421	0.678	0.593	0.232	1.299
r	0.425	0.106	0.046	0.045	0.110
S_r	0.1518	0.0379	0.0164	0.0162	0.0392
RSD_r (%)	4.4	5.6	2.8	7.0	3.0
R	2.591	0.568	0.489	0.208	0.969
S_R	0.9255	0.2027	0.1747	0.0743	0.3462
RSD_R (%)	27.1	29.9	29.5	32.1	26.7

Table A.5—Ethanol (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	0	1	1	1	1
Mean (mg/kg)	51.519	0.345	10.249	4.558	13.663
r	16.087	0.431	1.451	0.603	2.107
S_r	5.745	0.154	0.518	0.215	0.752
RSD_r (%)	11.2	44.6	5.1	4.7	5.5
R	39.158	0.517	2.305	1.660	6.585
S_R	13.985	0.185	0.823	0.593	2.352
RSD_R (%)	27.1	53.5	8.0	13.0	17.2

Table A.6—Ethanol (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	0	2	0	0	2
Mean (mg/kg)	45.892	0.460	13.182	7.102	17.138
r	11.424	0.164	2.001	1.248	1.146
S_r	4.0800	0.0584	0.7146	0.4458	0.4091
RSD_r (%)	8.9	12.7	5.4	6.3	2.4
R	58.019	0.686	15.141	9.196	19.924
S_R	20.7210	0.2450	5.4075	3.2843	7.1159
RSD_R (%)	45.2	53.3	41.0	46.2	41.5

Table A.7—Ethyl propanoate (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	1	1	1	1	0
Mean (mg/kg)	0.011	0.028	0.014	0.013	0.038
r	0.011	0.022	0.008	0.005	0.023
S_r	0.004	0.008	0.003	0.002	0.008
RSD_r (%)	34.9	28.6	20.2	14.7	22.1
R	0.047	0.092	0.040	0.034	0.102
S_R	0.017	0.033	0.014	0.012	0.037
RSD_R (%)	150.3	117.8	98.8	92.1	97.4

Table A.8—Ethyl propanoate (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	2	1	2	2	2
Mean (mg/kg)	0.009	0.031	0.004	0.005	0.020
r	0.003	0.012	0.004	0.006	0.004
S_r	0.0012	0.0042	0.0015	0.0021	0.0013
RSD_r (%)	14.2	13.5	36.5	44.9	6.6
R	0.020	0.139	0.014	0.013	0.039
S_R	0.0071	0.050	0.0052	0.0047	0.0140
RSD_R (%)	82.7	158.4	121.8	102.7	70.3

Table A.9—Hexanal (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	1	1	1	0	0
Mean (mg/kg)	0.457	0.954	2.265	1.786	1.939
r	0.138	0.067	0.254	0.257	0.298
S_r	0.049	0.024	0.091	0.092	0.107
RSD_r (%)	10.8	2.5	4.0	5.1	5.5
R	0.259	1.457	3.594	1.780	1.715
S_R	0.093	0.520	1.284	0.636	0.612
RSD_R (%)	20.3	54.5	56.7	35.6	31.6

Table A.10—Hexanal (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	0	1	1	1	1
Mean (mg/kg)	0.584	0.584	1.379	1.494	1.784
r	0.098	0.117	0.247	0.095	0.1303
S_r	0.0351	0.0419	0.0880	0.0338	0.0465
RSD_r (%)	6.0	7.2	6.4	2.3	2.6
R	0.547	0.555	1.057	1.261	1.552
S_R	0.1952	0.1982	0.3776	0.4505	0.5543
RSD_R (%)	17.3	33.9	27.4	30.1	31.1

Table A.11—3-Methyl-1-butanol (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	0	1	0	0	0
Mean (mg/kg)	0.507	0.286	0.242	0.128	1.048
r	0.044	0.040	0.029	0.029	0.116
S_r	0.016	0.014	0.010	0.01	0.041
RSD_r (%)	3.1	5.0	4.3	8.1	4.0
R	0.184	0.185	0.370	0.173	0.824
S_R	0.066	0.066	0.132	0.062	0.294
RSD_R (%)	12.9	23.2	54.6	48.5	28.1

Table A.12—3-Methyl-1-butanol (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	0	2	3	1	1
Mean (mg/kg)	0.425	0.215	0.150	0.076	0.877
r	0.054	0.029	0.013	0.020	0.090
S_r	0.0194	0.0102	0.0045	0.0070	0.0320
RSD_r (%)	4.6	4.7	3.0	9.3	3.7
R	0.444	0.379	0.297	0.161	0.929
S_R	0.1587	0.1354	0.1060	0.0576	0.3319
RSD_R (%)	37.4	63.0	70.8	76.1	37.8

Table A.13—(*E*)-2-Hexenal (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	1	1	0	0	1
Mean (mg/kg)	0.111	2.619	12.092	3.222	3.875
r	0.030	0.355	1.348	0.291	0.306
S_r	0.011	0.127	0.482	0.104	0.109
RSD_r (%)	9.6	4.8	4.0	3.2	2.8
R	0.193	2.315	7.282	2.697	5.289
S_R	0.069	0.827	2.601	0.963	1.889
RSD_R (%)	61.9	31.6	21.5	29.9	48.7

Table A.14—(*E*)-2-Hexenal (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	2	0	0	0	1
Mean (mg/kg)	0.137	2.746	10.140	3.211	4.714
r	0.019	0.421	1.186	0.383	0.305
S_r	0.0067	0.1504	0.4236	0.1366	0.1091
RSD_r (%)	4.9	5.5	4.2	4.3	2.3
R	0.248	2.396	8.599	2.817	4.056
S_R	0.0887	0.8559	3.0712	1.0059	1.4486
RSD_R (%)	64.7	31.2	30.3	31.3	30.7

Table A.15—(*Z*)-3-Hexenyl acetate (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	0	1	1	0	0
Mean (mg/kg)	0.645	1.753	0.641	0.598	0.722
r	0.117	0.175	0.119	0.100	0.183
S_r	0.042	0.063	0.042	0.036	0.065
RSD_r (%)	6.5	3.6	6.6	6.0	9.1
R	0.900	1.734	0.781	0.870	0.863
S_R	0.322	0.619	0.279	0.311	0.308
RSD_R (%)	49.9	35.3	43.5	52.0	42.7

Table A.16—(*Z*)-3-Hexenyl acetate (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	0	0	1	1	1
Mean (mg/kg)	0.810	2.079	0.520	0.666	0.795
r	0.217	0.481	0.106	0.107	0.071
S_r	0.0775	0.1719	0.0379	0.0382	0.0253
RSD_r (%)	9.6	8.3	7.3	5.7	3.2
R	0.974	2.526	0.476	0.736	0.803
S_R	0.3477	0.9021	0.1701	0.2629	0.2869
RSD_R (%)	42.9	43.4	32.7	39.5	36.1

Table A.17—(*E*)-2-Heptenal (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	1	1	1	1	0
Mean (mg/kg)	0.231	0.103	0.296	0.442	0.577
r	0.068	0.057	0.046	0.082	0.104
S_r	0.024	0.020	0.016	0.029	0.037
RSD_r (%)	10.6	19.8	5.5	6.6	6.5
R	0.377	0.187	0.829	0.723	0.990
S_R	0.134	0.067	0.296	0.258	0.353
RSD_R (%)	58.3	64.8	100.0	58.4	61.2

Table A.18—(*E*)-2-Heptenal (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	0	3	0	1	1
Mean (mg/kg)	0.194	0.075	0.161	0.298	0.376
r	0.043	0.022	0.058	0.050	0.031
S_r	0.0152	0.0080	0.0206	0.0179	0.0113
RSD_r (%)	7.8	10.7	12.8	6.0	3.0
R	0.365	0.157	0.344	0.562	0.710
S_R	0.1305	0.0560	0.1227	0.2006	0.2535
RSD_R (%)	67.2	74.2	76.0	67.3	67.4

Table A.19—6-Methyl-5-hepten-2-one (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	1	1	1	1	1
Mean (mg/kg)	0.059	0.059	0.080	0.068	0.069
r	0.017	0.028	0.022	0.018	0.015
S_r	0.006	0.010	0.008	0.007	0.006
RSD_r (%)	10.6	17.0	10.0	9.6	8.0
R	0.045	0.050	0.081	0.059	0.088
S_R	0.016	0.018	0.029	0.021	0.032
RSD_R (%)	27.1	29.8	36.3	30.9	45.9

Table A.20—6-Methyl-5-hepten-2-one (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	2	0	0	0	2
Mean (mg/kg)	0.051	0.048	0.068	0.058	0.047
r	0.009	0.018	0.019	0.019	0.007
S_r	0.0033	0.0065	0.0069	0.0067	0.0024
RSD_r (%)	6.6	13.5	10.2	11.4	5.2
R	0.111	0.089	0.158	0.121	0.091
S_R	0.0395	0.0317	0.0564	0.0433	0.0324
RSD_R (%)	78.2	66.2	82.7	74.4	69.3

Table A.21—1-Hexanol (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	1	1	1	1	0
Mean (mg/kg)	0.514	1.727	1.403	0.579	1.121
r	0.085	0.154	0.140	0.056	0.161
S_r	0.030	0.055	0.050	0.020	0.057
RSD_r (%)	5.9	3.2	3.6	3.5	5.1
R	0.764	1.915	2.352	1.106	1.652
S_R	0.273	0.684	0.840	0.395	0.590
RSD_R (%)	53.1	39.6	59.9	68.2	52.6

Table A.22—1-Hexanol (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	2	0	0	0	2
Mean (mg/kg)	0.530	1.763	1.298	0.532	1.103
r	0.027	0.264	0.164	0.088	0.060
S_r	0.0096	0.0943	0.0584	0.0315	0.022
RSD_r (%)	1.8	5.3	4.5	5.9	2.0
R	0.663	1.874	1.629	0.706	1.260
S_R	0.2367	0.6693	0.5819	0.2521	0.4499
RSD_R (%)	44.7	38.0	44.8	47.4	40.8

Table A.23—Nonanal (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	0	1	0	0	0
Mean (mg/kg)	2.533	0.546	1.064	0.606	2.886
r	0.793	0.156	0.298	0.150	1.057
S_r	0.283	0.056	0.107	0.054	0.378
RSD_r (%)	11.2	10.2	10.0	8.8	13.1
R	3.520	0.940	1.653	0.942	5.157
S_R	1.257	0.336	0.590	0.336	1.842
RSD_R (%)	49.6	61.5	55.5	55.5	63.8

Table A.24—Nonanal (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	0	2	2	2	1
Mean (mg/kg)	2.839	0.911	1.022	0.648	2.908
r	1.134	0.176	0.496	0.130	0.386
S_r	0.4048	0.0627	0.1772	0.0464	0.1379
RSD_r (%)	14.3	6.9	17.3	7.2	4.7
R	5.321	1.738	1.716	1.016	3.747
S_R	1.9003	0.6207	0.6129	0.3627	1.3383
RSD_R (%)	66.9	68.1	60.0	55.9	46.0

Table A.25—1-Octen-3-ol (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	1	1	1	1	1
Mean (mg/kg)	0.029	0.017	0.026	0.112	0.040
r	0.025	0.014	0.007	0.005	0.015
S_r	0.009	0.005	0.002	0.002	0.006
RSD_r (%)	32.6	27.8	9.1	1.7	14.3
R	0.084	0.043	0.057	0.657	0.093
S_R	0.030	0.015	0.020	0.235	0.033
RSD_R (%)	108.9	87.4	79.9	208.6	86.0

Table A.26—1-Octen-3-ol (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	2	2	1	1	2
Mean (mg/kg)	0.025	0.018	0.023	0.030	0.039
r	0.008	0.004	0.010	0.012	0.007
S_r	0.0029	0.0013	0.0035	0.0042	0.0025
RSD_r (%)	11.9	7.5	15.4	13.9	6.5
R	0.030	0.023	0.039	0.048	0.060
S_R	0.0106	0.0082	0.0140	0.0170	0.0215
RSD_R (%)	43.0	45.8	61.6	56.1	55.7

Table A.27—(E,E)-2,4-Hexadienal (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	1	0	0	0	1
Mean (mg/kg)	0.202	1.039	1.883	0.395	0.500
r	0.134	0.413	0.210	0.075	0.091
S_r	0.048	0.148	0.075	0.027	0.032
RSD_r (%)	23.0	14.4	4.0	6.8	6.4
R	0.658	3.645	6.562	1.100	1.111
S_R	0.235	1.302	2.344	0.392	0.397
RSD_R (%)	113.2	126.6	125.7	99.0	79.0

Table A.28—(E,E)-2,4-Hexadienal (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	3	3	2	3	0
Mean (mg/kg)	0.020	0.112	0.219	0.101	0.294
r	0.019	0.034	0.079	0.034	0.153
S_r	0.0069	0.0123	0.0282	0.0123	0.0547
RSD_r (%)	35.4	11.0	12.9	12.1	18.6
R	0.078	0.292	0.486	0.227	0.999
S_R	0.0278	0.1041	0.1736	0.0810	0.3568
RSD_R (%)	142.4	93.3	79.3	80.2	121.6

Table A.29—Acetic acid (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	1	1	0	0	1
Mean (mg/kg)	10.893	8.487	3.521	1.790	3.215
r	2.936	0.501	0.502	0.207	0.266
S_r	1.049	0.179	0.179	0.074	0.095
RSD_r (%)	9.6	2.1	5.1	4.1	3.0
R	8.062	4.825	2.268	1.173	2.931
S_R	2.879	1.723	0.810	0.419	1.047
RSD_R (%)	26.4	20.3	23.0	23.4	32.6

Table A.30—Acetic acid (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	2	1	3	2	1
Mean (mg/kg)	10.402	7.935	3.141	1.515	3.755
r	2.537	1.053	0.397	0.102	0.330
S_r	0.9060	0.3761	0.1417	0.0363	0.1180
RSD_r (%)	8.7	4.7	4.5	2.4	3.1
R	12.674	8.715	3.136	1.500	4.832
S_R	4.5263	3.1125	1.1200	0.0535	1.7258
RSD_R (%)	43.5	39.2	35.7	35.4	46.0

Table A.31—Propanoic acid (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	0	0	1	0	1
Mean (mg/kg)	0.366	0.229	0.162	0.221	0.537
r	0.038	0.060	0.020	0.054	0.066
S_r	0.013	0.022	0.007	0.019	0.024
RSD_r (%)	3.7	9.4	4.4	8.6	4.4
R	0.262	0.245	0.278	0.270	0.425
S_R	0.094	0.088	0.099	0.096	0.152
RSD_R (%)	25.5	38.3	61.1	43.6	28.3

Table A.32—Propanoic acid (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	0	0	2	2	1
Mean (mg/kg)	0.310	0.165	0.100	0.124	0.476
r	0.052	0.063	0.017	0.013	0.048
S_r	0.0185	0.0226	0.0062	0.0046	0.0173
RSD_r (%)	6.0	13.6	6.2	3.7	3.6
R	0.665	0.317	0.225	0.244	1.023
S_R	0.2376	0.1132	0.0802	0.0873	0.3655
RSD_R (%)	76.8	68.4	80.4	70.3	76.8

Table A.33—(*E*)-2-Decenal (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	1	1	0	0	1
Mean (mg/kg)	0.651	0.292	0.664	0.929	0.829
r	0.485	0.314	0.233	0.689	0.421
S_r	0.173	0.112	0.083	0.246	0.150
RSD_r (%)	26.6	38.5	12.6	26.5	18.1
R	1.218	1.187	1.466	1.994	1.795
S_R	0.435	0.424	0.523	0.712	0.641
RSD_R (%)	66.8	145.2	78.8	76.6	77.3

Table A.34—(*E*)-2-Decenal (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	0	0	0	1	1
Mean (mg/kg)	0.994	0.295	0.522	0.669	0.858
r	1.091	0.257	0.202	0.198	0.247
S_r	0.3898	0.0917	0.0722	0.0707	0.0880
RSD_r (%)	39.2	31.1	13.8	10.6	10.3
R	2.633	0.827	1.541	1.340	2.163
S_R	0.9405	0.2952	0.5503	0.4784	0.7724
RSD_R (%)	94.6	100.1	105.5	71.5	90.0

Table A.35—Pentanoic acid (mg/kg) by SPME-GC-FID.

Sample	A	B	C	D	E
n	8	8	8	8	8
Outliers	0	1	1	0	1
Mean (mg/kg)	0.084	0.071	0.051	0.073	0.080
r	0.048	0.013	0.028	0.063	0.012
S_r	0.017	0.005	0.010	0.022	0.004
RSD_r (%)	20.2	6.3	20.0	30.6	5.4
R	0.191	0.140	0.110	0.158	0.152
S_R	0.068	0.050	0.039	0.056	0.054
RSD_R (%)	80.9	70.3	77.5	76.8	68.0

Table A.36—Pentanoic acid (mg/kg) by SPME-GC-MS.

Sample	A	B	C	D	E
n	12	12	12	12	12
Outliers	1	2	0	1	1
Mean (mg/kg)	0.049	0.038	0.038	0.041	0.062
r	0.033	0.006	0.022	0.007	0.007
S_r	0.0116	0.0022	0.0079	0.0026	0.0027
RSD_r (%)	23.7	5.7	20.8	6.2	4.3
R	0.114	0.097	0.093	0.110	0.133
S_R	0.0406	0.0347	0.0331	0.0392	0.0476
RSD_R (%)	82.8	91.1	87.1	94.6	77.0