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Food Chemistry 90 (2005) 69–79
Food Chemistry
www.elsevier.com/locate/foodchem

Comparative study on volatile analysis of extra virgin olive oil by dynamic headspace and solid phase micro-extraction

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Received 15 January 2004; received in revised form 15 March 2004; accepted 18 March 2004

Abstract

The dynamic headspace thermal desorption (DHS-TD) apparatus using Tenax-TA and Carbotrap-300 traps, connected to a gas chromatography (GC)-ion trap-mass spectrometry (MS) equipment, as well as the solid phase micro-extraction (SPME) tool, with polydimethylsiloxane (PDMS) and polydimethylsiloxane-divinylbenzene (PDMS-DVB) fibers and connected to a GC-time of flight-MS equipment were implemented for the isolation and identification of virgin olive oil volatile compounds under various sampling conditions. Applying the DHS-TD Tenax-TA procedure separated a higher number of compounds compared to the SPME-PDMS-DVB, which on the other hand required shorter total times for the analysis. High ratio of gas flow rate/amount of oil gave better results for DHS-TD, while a high ratio of headspace/amount of oil worked better for SPME. PDMS exhibited a low sensitivity to olive oil polar volatile compounds while PDMS-DVB showed the overall best sensitivity for all classes of volatile compounds. Results indicate that SPME may find a wide application as an analytical technique for quick analysis of quality related volatile compounds of olive oil. The analyses performed on the GC-TOF-MS-system demonstrated high sensitivity and also high selectivity due to the high quality of mass spectra obtained. The SPME-GC/TOFMS technique appears to be faster and simpler than DHS-TD/GC/MS but the latter provides higher efficiency.

Keywords: Olive oil; Purge and trap; Volatile compounds; Solid phase micro-extraction; SPME; Analysis

1. Introduction

Virgin olive oil, which is obtained from fresh and mature fruit of the olive tree *Olea europeae* has a unique volatile composition contributing to its aroma and flavor (Kiritsakis, 1998; Kiritsakis & Christie, 2000). Several researchers (Aparicio & Morales, 1998; Fedeli, 1977; Flath, Forrey, & Guadagni, 1973; Kiritsakis, 1998) reported the chemical identity of a great number of volatile compounds in olive oil. Analysis of the aroma volatile compounds has been used to evaluate the degree of ripeness of the olive fruit (Aparicio & Morales, 1998). According to Kiritsakis (1998) and Salas and Sanchez (1999), methods used and conditions applied to obtain olive oil from olive fruit affect its volatile composition. The malaxation (mixing) process during olive fruit processing in an olive oil mill affects evolution of olive oil volatiles (Angerosa, d'Alessandro, Basti, & Vito, 1998).

Various attempts to quantify these compounds have been reported. Guth and Grosch (1993) and Reiners and Grosch (1998) used the stable-isotope dilution analysis. Dynamic headspace-thermal desorption combined to a GC (DHS-TD/GC) has been a very popular technique proven for its performance and widely used in a great number of studies (Ahn, Jo, & Olson, 1999; Aparicio & Morales, 1994; Morales, Aparicio, & Rios, 1994; Sucan, Fritz-Jung, & Ballan, 1998) for the isolation of the olive oil flavor volatile compounds. However, not as much

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doi:10.1016/j.foodchem.2004.03.025

information has been available on the optimization of this technique in terms of purging time and temperature conditions.

Introduce din the early ''90s, solid phase micro-extraction (SPME) has since been used among others for the aroma analysis of various food products such as fruit (Song, Gardner, Holland, & Beaudry, 1997), alcoholic beverages (Ng, Hupe, Harnois, & Moccia, 1996), coffee (Roberts, Pollien, & Milo, 2000) and microorganism metabolites (Vergnais, Masson, Montel, Berdague, & Talon, 1998). Lately, the SPME technique has been applied for the analysis of flavors from lipid and oil containing products (Jelen, Obuchowska, Zawirska-Wojtasiak, & Wasowicz, 2000; Snyder, King, & Zhang, 1998). For a rapid and accurate aroma compound characterization of the different products, time-of-flight mass spectrometry (TOF-MS) (Song et al., 1997), isotope ratio mass spectrometry (IRMS) (Gupry, Rochut, Robins, & Gentil, 2000), and gas chromatography/olfactometry dilution analysis (Deibler, Acree, & Lavin, 1999) have been combined with the SPME. Since the SPME analysis techniques could provide quick and accurate results (Song et al., 1997) it has been used lately in the analysis of the unique volatile spectra of olive oil. A rather more thorough investigation on the optimization of the analysis and a better understanding of its benefits and liability could be of great value in order to further understand and improve the extraction process.

This work was designed to optimize the operation conditions, and evaluate the efficiency of the dynamic headspace thermal desorption and solid phase microextraction analytical techniques, in the analysis of volatile compounds of olive oil. A suggestion of application guidelines towards a more efficient utilization of these techniques was also attempted.

2. Materials and methods

One-liter glass bottled commercial, cold-processed Spanish extra virgin olive oil (Aceites del Sur S.A., Spain), was purchased from a local market in East Lansing, MI. Chemical analytical evaluation of oil was not run, trusting the labeling information.

For the dynamic headspace thermal desorption the Carbotrap-300 and Tenax-TA traps of 11.5 cm × 6 mm dimensions (Supelco, Bellefonte, PA) were used, after preconditioning for 8 h at 350 °C and purging for 30 min with nitrogen gas, prior to use. Stripping of volatile compounds from olive oil, placed in 250 ml GWB with a fritted dispersion glass tube, was performed by the dynamic headspace trapping and desorption technique. Dry nitrogen gas was bubbled into the bottles containing either 80, 160 or 240 g oil. The applied gas flow rates were either 100 or 200 ml/min, while the stripping times were 10, 20, 40, 80, or 100 min. During the stripping process GWB remained in a water bath at 37 °C while the fitted glass traps at the outlet of the bottles were maintained at 23 °C. The stripping temperature of 37 °C was chosen to avoid thermal volatiles alterations, although more compounds would be possible stripped if oil samples were exposed to temperatures up to 50 °C (Reiners & Grosch, 1998). A Dymatherm 1000 (Dynatherm Analytical Instruments Inc., Kelton, PA) apparatus was used as another means for stripping oil volatiles in traps for further analysis with the DHS-TD technique. Helium gas was used as a stripping gas. Oil samples of 1, 3 and 6 g were placed in the 9 ml glass-stripping receptacle. Samples were preheated for 5 min at 37 °C and purged for 15 or 30 min applying 100 or 200 ml/min flow rate. Dry helium flowed through the traps for 1 min at 25 ml/min to remove any possible moisture. All analysis were performed at least in triplicate.

The desorption of the compounds stripped from the oil placed in both GWB and Dynatherm apparatus and retained by the traps, was performed by a Dynatherm desorption unit Model 890. The desorption unit was connected to a gas chromatography by a transfer line. Helium at 7 ml/min and 750 kPa was flowing to desorb the molecules from the trap and convey them into the chromatographic column. During desorption traps were kept at 300 °C for 8 min. All the transfer lines and the valves were maintained at 230 °C to avoid condensation of volatiles. Before using the traps again, they were cleaned by heating them for 30 min at 320 °C while purged with helium.

The chromatographic analysis for the DHS-TD technique implemented a Hewlett Packard 5890 Series II GC (Hewlett Packard, Philadelphia, PA) with a 30 m × 0.32 mm ID × 0.25 μm film thickness, fused silica SPB-5, capillary column from Supelco (Bellefonte, PA). A FID detector was used for quantifying the volatile compounds separated by both GWB and Dynatherm apparatus. Integration of chromatography peaks was performed using a Hewlett Packard HP 3395 integrator, (Hewlett Packard, Philadelphia, PA). For avoiding saturation of the GC detector by the volatile compounds, the amount of the oil and the purging time, were balanced. During striping of 160 and 240 g of oil at 200 ml/min gas flow rate for a long stripping time (80–100 min), two events were commonly occurring. The one was related to the sampling column overload which could be concluded by the end tailing of the peaks in the relevant chromatograms. The second event was the saturation of the FID detector recorded through the signal failure during the analysis. Both events may be indicative of the fact that the trapping material could collect enough volatile compounds, therefore, a poor chromatographic separation would be accomplished. Conditions used for the analysis were: initial temperature, 35 °C for 1 min, increased to 80 °C at a rate of 3 °C/min, held for 1 min,

then increased to 180 °C at 10 °C/min, held for 1 min, and finally up to 260 °C at 4 °C/min where held for 10 min. Carrier gas was Helium at 88 kPa (8 psi) flowed at 1.75 ml/min at 40 °C. Calibration curves were obtained by injecting known amounts of standard compounds in the gas-washing bottle. Selected standard compounds were hexanal, 2-hexenal, nonanal, 2-nonenal, 2-nonanone, acetaldehyde, ethyl butyrate, methylbutanal, hexene, octanal, 2,4-decadienal, butanal, pentanal, 1-pentanol, heptanal, 2-decenal, 2-heptenal decanal, 2-butylfuran, hexyl acetate and 3-pentanone (Sigma–Aldrich, St. Louis, MO, USA).

Ion trap-mass spectrometry (ITMS) was used to identify compounds from the extra virgin olive oil retained in the traps by the DHS-TD procedure. A Varian 2000 mass spectrometer (Varian, TX, USA) was interfaced with the Dynatherm desorption unit operating at the aforementioned conditions. Tuning values for the ITMS were 100 using cedrol as tuning standard. Tune sensitivity: 9000. Acquisition parameters: full scan, scan range: 41–300 amu, scan time: 1.0 s, threshold: 1 count, multiplier from 1500 to 2300 V depending on multiplier conditions. Transfer line temperature 240 °C, exit nozzle, 240 °C, manifold 240 °C.

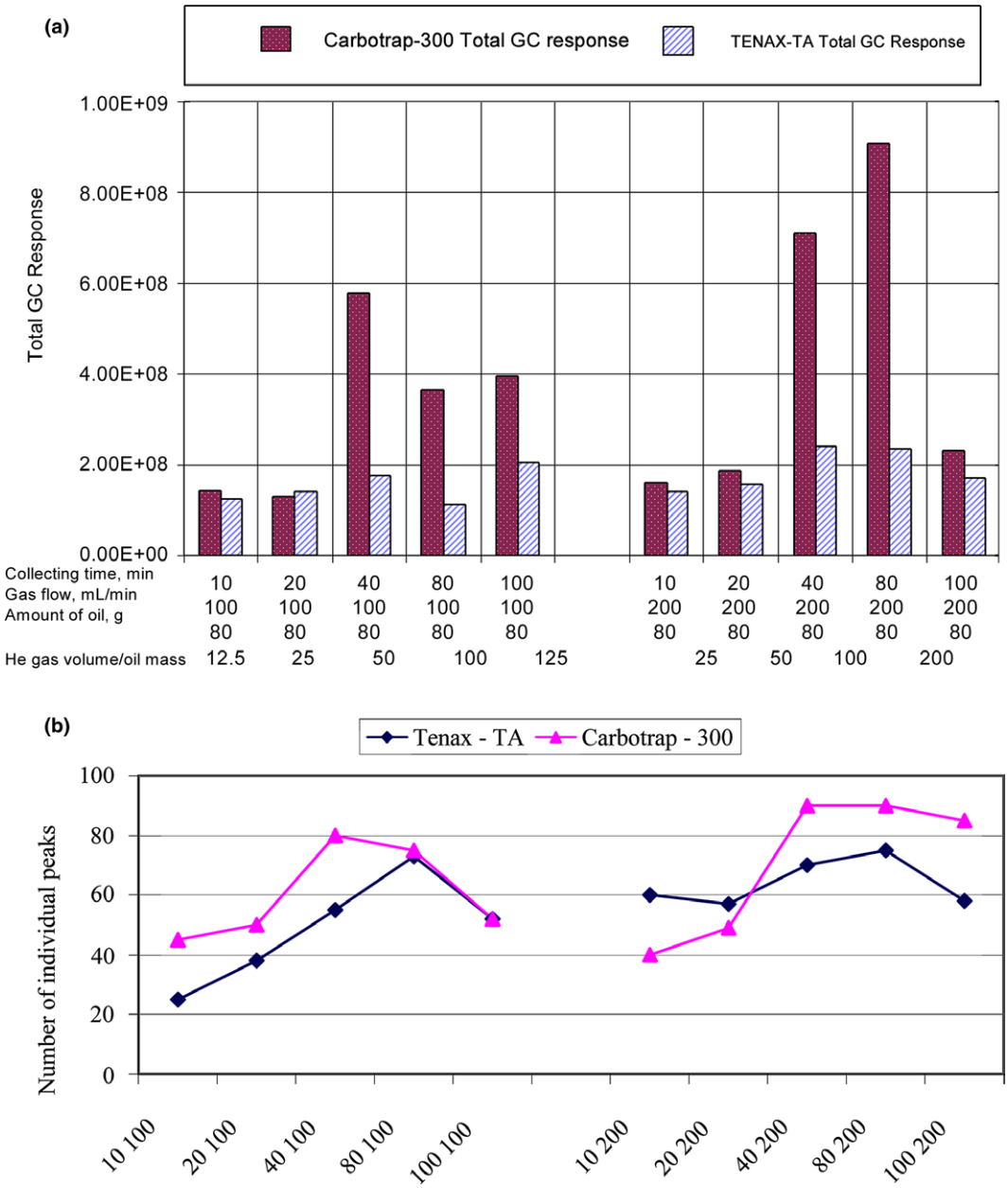

Fig. 1. Comparing Carbotrap-300 to Tenax-TA traps in volatile analysis of virgin olive oil using DHS-TD and glass washing bottles for different periods and flow rates. (a) Total gas chromatography response and (b) total number of peaks isolated.

SPME procedure was performed using the polydimethylsiloxane (PDMS) 1 cm long–100 μm phase thickness, and the polydimethylsiloxane-divinylbenzene (PDMS-DVB) 1 cm long-65 μm fibers (Supelco, Bellefonte, PA). Fibers were preconditioned at 250 °C for 1 h. During sampling fibers were exposed for 10, 20, 30, and 40 min to the headspace of tightly closed 12 ml glass vials containing 3.5, 7, or 10.5 g of olive oil. Before sampling, the vials were allowed to equilibrate for 2, 4 and 6 h at 23 °C. Sampling was performed at the same temperature. Rubber septum closures with a Teflon liner were used to close the vials. No agitation or stirring were applied to the vials during equilibrium or sampling. All analysis were performed at least in triplicate.

Desorption of volatile compounds from the SPME fibers was performed by placing the fibers into the injection port of the GC and exposing them to a helium flow at 250 °C for 2 min. Volatiles were cold trapped by liquid nitrogen at the beginning of the GC column. At the end of the 2 min period the fibers were retracted out of the injection port, the liquid nitrogen was removed, the GC door closed and the chromatographic program was initiated.

The gas chromatography-time-of-flight mass spectrometry (GC-TOFMS) was used to separate and identify the compounds isolated by SPME. A Hewlett-Packard HP-6890 gas chromatography (Hewlett Packard, Philadelphia, PA), equipped with a fused silica capillary column HP-5, (Hewlett Packard, Philadelphia, PA), 5.00 m × 0.1 mm ID., and 0.34 mm coating thickness was used. Carrier gas was 99.999% purity helium flowing at a rate of 0.5 ml/min. Temperature program was: 1.5 min at 35 °C, raised to 250 °C at 50 °C/min and held for 2 min. The GC/MS transfer line was kept at 220 °C. The detection and identification of volatile compounds was carried out in a Leco (Leco Inc., St. John, MI, USA) electron impact ionization Time of Flight detector model FCD-650. Mass spectra were collected at a rate of 40 spectra/s over a range of m/z 40–300 with ionization energy of 70 eV.

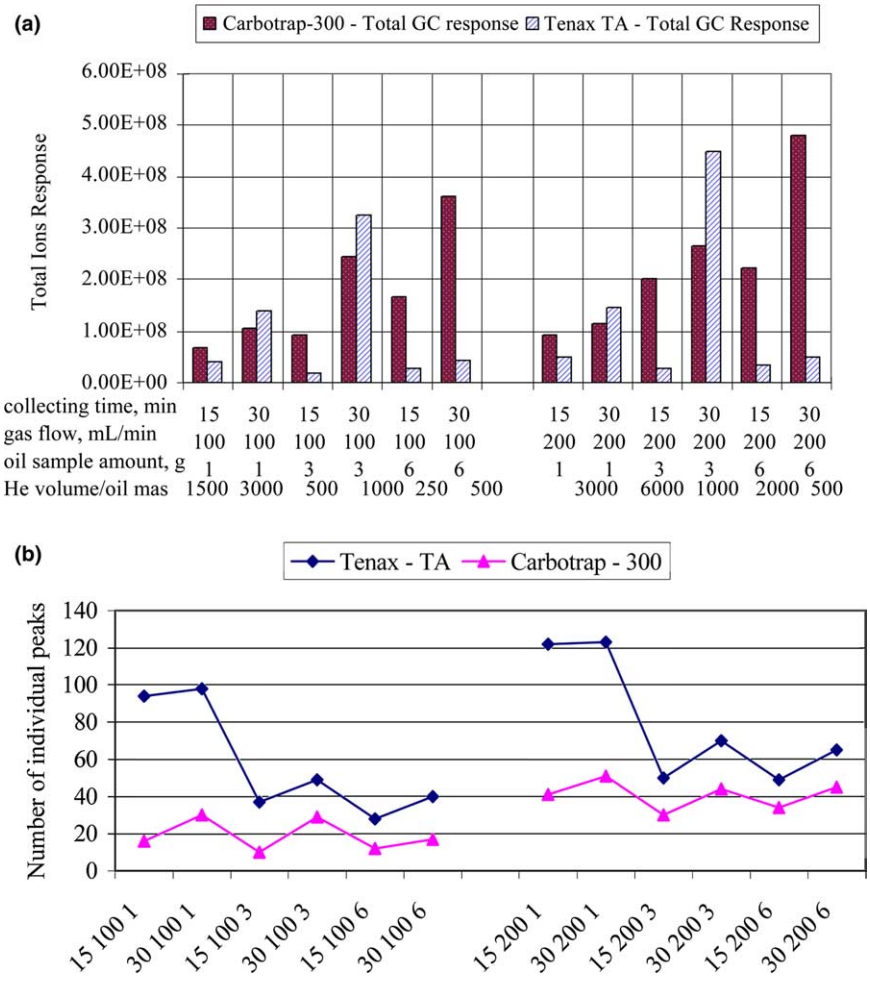

Fig. 2. Total isolated volatile compounds from olive oil in 9 ml vials at two flow rates and collecting periods using DHS-TD-Carbotrap-300 and Tenax-TA traps. (a) Total gas chromatography response and (b) total number of peaks isolated.

3. Results

3.1. Dynamic headspace thermal desorption

In order to optimize the extraction and trapping conditions using the GWB and Dynatherm for the two trapping materials (Tenax-TA and Carbotrap-300), different flow rates, amounts of oil, and stripping times were evaluated. Data from the analytical results are compared in terms of both the number of peaks and amount of compounds (total GC response), which in this work is referred to as *analytical efficiency* of the method. Fig. 1 shows the isolation conditions of the volatile compounds using GWB extraction for this study. The optimum conditions were: 200 ml/min of He, 80 g of olive oil, and 80 min of collecting period. Carbotrap-300 yielded approximately 90 peaks while 75 peaks were recorded for Tenax-TA (Fig. 1b). As shown, Carbotrap-300 is more efficient than Tenax-TA at all the applied conditions. Higher values were obtained using the 200 ml/min flow rate of He than at 100 ml/min. There appears to be high efficiency conditions to stripping times within the range of 40–80 min. Above this time range the efficiency decreased, probably because at higher stripping times compounds started to desorb from the trap, counterbalancing those already sorbed by it.

Results for the Dynatherm apparatus using two flow rates, two collecting periods and three amounts of oil, for both Tenax-TA and Carbotrap-300, are shown in Fig. 2. The highest number of peaks was recorded with 200 ml/min flow rate as compared to the 100 ml/min, for both traps (Fig. 2b). For each flow rate, the highest number of peaks corresponded to the highest ratio of He volume/oil mass. However, the total GC response increased as the He volume/oil mass ratio decreased (Fig. 2a). In general, the stripping efficiency was higher for 30 min collecting time than for 15 min.

Tables 1 and 2 present selected isolated compounds (as a function of retention time) and the GC percent area responses for different stripping conditions. When Tenax-TA was used in the Dynatherm apparatus and the stripping time was increased from 15 to 30 min, a greater quantity of low volatile compounds such as 2-decenal, 2-undecenal and 2-heptenal was markedly increased, while for other compounds such as nonanal, hexanol, hexanal, ethyl-2-methylbutyrate (Table 1) the quantities remained similar. This may indicate that a higher He volume/oil mass ratio could increase the amount of larger molecular weight volatile compounds

Table 1
Volatile compounds of virgin olive oil isolated by DHS-TD using Dynatherm-Tenax-TA at different conditions

Compound name	RT (min)	GC area percent					
		1a	1b	1c	1d	1e	1f
Acetic acid	1.57	0.37	9.75	1.57	0.14	0.2	0.11
Ethyl-2-methyl-butyrate	3.95	8.24	1.06	3.95	22.02	30.05	26.2
3-Methyl-butanol	4.5	1.66	3.58	4.5	0.98	0.84	0.71
4-Methyl-2-methyl-butanol	4.75	0.23	0.035	4.75	0.9.7	0.5	0.55
Ethyl iso butyrate	5.05	0.16	2.9	5.05	0.9	0.28	0.83
1-Penten-3-one	5.58	1.13	0.04	5.58	0.36	7.98	0.32
Hexanal	6.1	0.94	1.64	6.1	15.66	0.005	14.21
(Z)-3-hexenal	7.0	0.037	0.05	7.0	0.1	5.83	0.1
(E)-2-hexenal	7.6	0.04	N.D.	7.6	9.9	0.499	9.04
(Z)-3-hexenol	7.75	0.35	N.D.	7.75	0.95	2.46	0.87
Hexanol	8.5	6.08	0.2	8.5	3.44	0.42	3.01
2-Heptenal	12.56	1.12	N.D.	12.56	0.086	0.11	0.064
a-Farnecene	13.02	0.78	0.99	13.02	0.19	0.05	0.146
Quaiacol	13.6	0.075	0.45	13.6	0.24	0.33	0.166
(Z)-3-hexyl acetate	14.28	1.53	0.12	14.28	0.21	N.D.	0.18
Nonanal	14.85	0.12	0.27	14.58	0.44	N.D.	0.34
2-Octenal	15.46	0.12	N.D.	15.46	N.D.	N.D.	0.14
2,4-Heptadienal	16.36	1.62	N.D.	16.36	0.43	0.1	0.36
2-Pentyl ethyl alcohol	18.8	0.1	1.67	18.8	0.5	N.D.	0.45
2-Decenal	19.6	3.84	0.56	19.6	0.04	0.08	N.D.
(Z)-2-nonenal	20.5	0.136	2.91	20.5	0.037	0.17	N.D.
2-Undecenal	21.42	0.4	1.16	21.42	0.3	N.D.	0.25
(E,E)-2,4-decadienal	24.0	0.69	0.21	24.0	N.D.	0.19	N.D.

1a: Percent area of isolated compounds from 1 g oil purged with 200 ml/min for 15 min according to retention times (min). He/oil ratio = 3000.
1b: Percent area of isolated compounds from 1 g oil purged with 200 ml/min for 30 min according to retention times (min). He/oil ratio = 6000.
1c: Percent area of isolated compounds from 3 g oil purged with 200 ml/min for 15 min according to retention times (min). He/oil ratio = 1000.
1d: Percent area of isolated compounds from 3 g oil purged with 200 ml/min for 30 min according to retention times (min). He/oil ratio = 2000.
1e: Percent area of isolated compounds from 6 g oil purged with 200 ml/min for 15 min according to retention times (min). He/oil ratio = 500.
1f: Percent area of isolated compounds from 6 g oil purged with 200 ml/min for 30 min according to retention times (min). He/oil ratio = 1000.

Table 2
Volatile compounds of virgin olive oil isolated by DHS-TD using Dynatherm-Carbotrap-300 at different conditions

Compound name	RT (min)	GC area percent					
		1a	1b	1c	1d	1e	1f
Acetic acid	1.57	0.27	0.014	0.008	0.032	0.03	0.085
Ethyl-2-methyl-butyrate	3.95	34.8	N.D.	33.9	N.D.	28.43	N.D.
3-Methyl-butanol	4.5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4-Methyl-2-methyl-butanol	4.75	1.46	0.2	N.D.	0.79	N.D.	0.47
Ethyl iso butyrate	5.05	N.D.	0.509	N.D.	1.11	N.D.	0.199
1-Penten-3-one	5.58	3.21	N.D.	1.48	N.D.	1.12	N.D.
Hexanal	6.1	0.86	2.33	1.24	4.53	0.018	1.44
(Z)-3-hexenal	7.0	1.96	N.D.	0.81	N.D.	0.82	N.D.
(E)-2-hexenal	7.6	0.19	1.33	0.08	2.9	N.D.	0.79
(Z)-3-hexenol	7.75	1.062	0.1	0.76	0.48	N.D.	0.058
Hexanol	8.5	N.D.	0.43	N.D.	1.55	N.D.	0.25
2-Heptenal	12.56	0.042	N.D.	0.016	N.D.	0.016	N.D.
a-Farnecene	13.02	0.021	0.022	N.D.	0.07	0.008	0.013
Quaiacol	13.6	0.109	0.029	0.033	0.073	0.033	0.018
(Z)-3-hexyl acetate	14.28	N.D.	0.028	N.D.	0.068	N.D.	0.016
Nonanal	14.85	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2-Octenal	15.46	N.D.	0.029	N.D.	0.071	N.D.	0.016
2,4-Heptadienal	16.36	0.034	0.051	0.011	0.13	0.01	0.03
2-Pentyl ethyl alcohol	18.8	N.D.	0.053	N.D.	0.22	N.D.	0.031
2-Decenal	19.6	0.042	N.D.	0.015	0.16	0.016	N.D.
(Z)-2-nonenal	20.5	0.067	N.D.	0.02	0.026	0.02	N.D.
2-Undecenal	21.42	N.D.	0038	N.D.	0.11	N.D.	0.022
(E,E)-2,4-decadienal	24.0	0.08	N.D.	0.03	N.D.	0.03	N.D.

1a: Percent area of isolated compounds from 1 g oil purged with 200 ml/min for 15 min according to retention times (min). He/oil ratio = 3000.
1b: Percent area of isolated compounds from 1 g oil purged with 200 ml/min for 30 min according to retention times (min). He/oil ratio = 6000.
1c: Percent area of isolated compounds from 3 g oil purged with 200 ml/min for 15 min according to retention times (min). He/oil ratio = 1000.
1d: Percent area of isolated compounds from 3 g oil purged with 200 ml/min for 30 min according to retention times (min). He/oil ratio = 2000.
1e: Percent area of isolated compounds from 6 g oil purged with 200 ml/min for 15 min according to retention times (min). He/oil ratio = 500.
1f: Percent area of isolated compounds from 6 g oil purged with 200 ml/min for 30 min according to retention times (min). He/oil ratio = 1000.

removed from the virgin olive oil. As Table 2 shows, Carbotrap-300 was not suitable for isolating compounds of low partial pressure and although higher stripping times (30 min) were applied, insignificant changes were recorded for most of the isolated compounds.

3.2. Extraction efficiency of gas wash bottles and Dynatherm processes

The extraction efficiency (in term of the number and amount of compounds isolated) of the GWB and the Dynatherm apparatuses was evaluated by flowing precise volumes of pure dry helium through various amounts of oil at different stripping times. The ratio "oil mass/total He gas volume" was calculated and correlated to the extraction efficiency. For the GWB, conditions were 80 ml of olive oil, 100 ml/min of gas He, and the following times were 10, 20, 40, 80 and 100 min. He gas volume/oil mass ratios were (in ml/g): 12.5, 25.0, 50.0, 100 and 125 respectively. For the 200 ml/min He gas flow, the oil mass/He volume ratios were: 25.0, 50.0, 100, 200, and 250 ml/g. For the Dynatherm apparatus, a He flow of 200 ml/min during 15 and 30 min for different amounts of oil was evaluated. For 1 ml of oil, the oil mass/He gas volume ratios were 3000 and 6000 ml/g; for 3 ml of oil the ratio values were 1000 and 2000; and for 6 ml of oil, 500 and 1000 ml/g; respectively for the two time periods.

Results showed that for the GWB, as the He volume/oil mass ratio increased in the range of 12.5–100 ml/g, the separation efficiency increased. However, less separation was achieved for higher volume/mass ratio such as 125 ml/g. This was probably due to excessive flowing times (about 100 min) that may desorb the compounds already in the trap (Fig. 1). Supportive evidence for that was the slight olfactory response when sniffing above the trap used at such conditions, while an attempt to trap the flavor compounds although not quite successful, did reveal some compounds (data not shown). Furthermore, identical He volume/oil mass ratios yielded a different number of peaks and amount of compounds (Figs. 1 and 2). The relative amounts of compounds isolated and identified from olive oil placed in GWB and Dynatherm 1000 are listed in Tables 1 and 2 according to their retention times. Isolated compounds appeared to differ in their GC area percentage depending on the particular values of He volume/oil mass ratios applied during stripping.

3.3. Solid phase micro-extraction

Factors affecting the performance of the SPME extraction process include the fiber characteristics; temperature; sampling time during the extraction process as well as the polar balance, molecular size, and relative amount of the analytes present in the sample. It has been reported that polar fibers show higher extraction capacity compared to non-polar one, in terms of the variety of the volatile compounds extracted from oil samples (Jelen et al., 2000), but to the best of our knowledge, there is a lack of information on the application of bipolar fibers to edible oil analysis. Selecting a proper sampling temperature is important, since it could provide the proper level of energy necessary to vaporize the analytes. Since different analytes have different energy requirements, temperature selection may significantly impact the concentration of the analytes in the headspace. On the other hand, since the retention of the compounds by the fiber is an exothermic process, high temperatures do not favor it. Zhang and Pawliszyn (1995) reported that different temperatures during sampling, resulted in different amounts of compounds being adsorbed in the SPME fibers. In the case of fat containing products, a careful selection of the sampling temperature is also needed in order to minimize the oxidation and avoid the breakdown of flavor precursors leading to the formation of off flavors (Saxby, 1993). The establishment of a more close comparison between the DHS-TD and the SPME techniques would have asked for the same sampling temperature for both techniques. The SPM-extraction though, is a static process mainly depended on the volatility of the compounds. Exposing the olive oil at 35 °C, the high

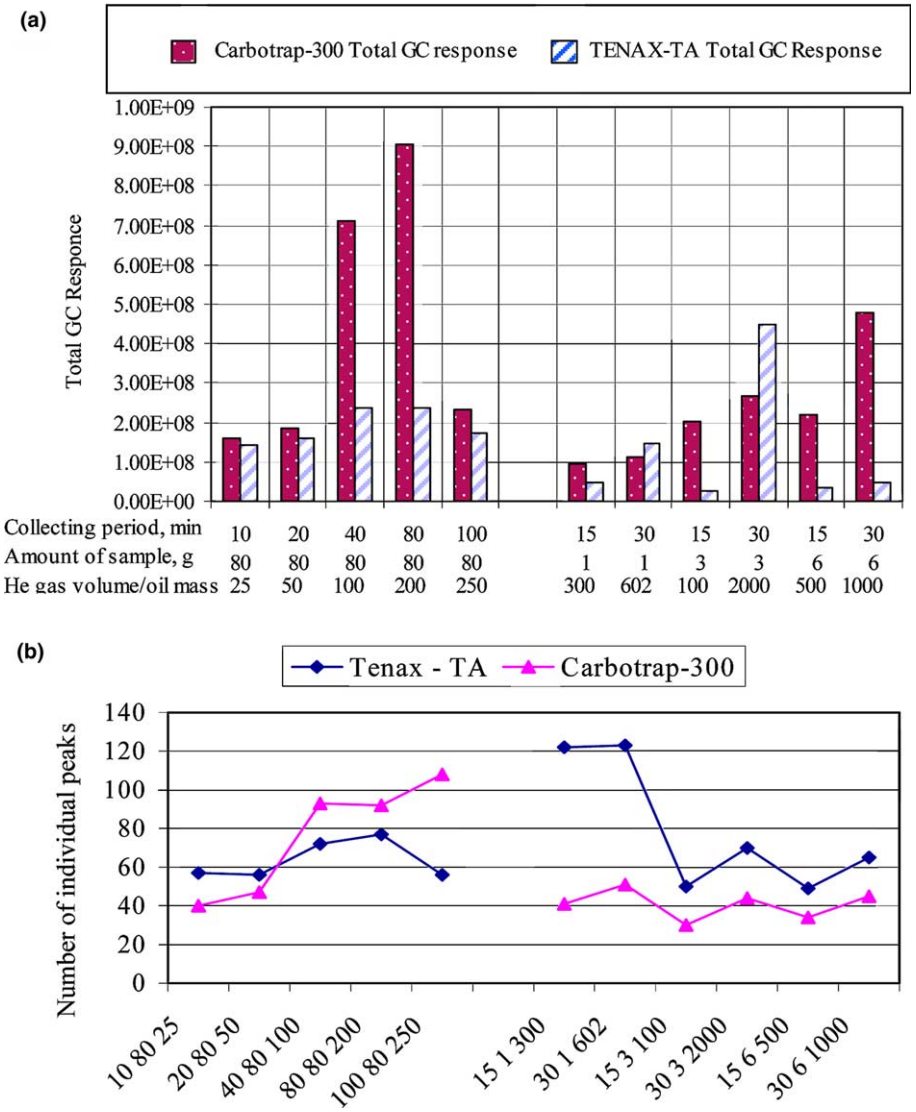

Fig. 3. Gas chromatography volatile analysis of virgin olive oil by DHS-TD technique using different combination of traps, purging times, flow rates and oil amounts. (a) Total gas chromatography response and (b) total number of peaks isolated.

volatile-low molecular weight compounds in the oil could evaporate, saturate the headspace and consequently occupy the limited available sides on the fiber excluding the rest of the compounds. Thus, sampling at that temperature would have created competitive thermodynamic conditions in the headspace between low and high volatile compounds that would have led to poorer extraction efficiency. In that sense, the temperature of 23 °C for the SPME analysis was selected.

3.4. Extraction efficiency of polydimethylsiloxane and polydimethylsiloxane-divinylbenzene fiber

The two types of fibers used, PDMS and PDMS/DVB, gave different GC responses, which can be attributed to their different polarity (Fig. 3a). Table 3 presents an overall picture of the olive oil compounds found in the relevant literature of Kiritsakis (1998), Aparicio and Morales (1998) and Morales et al. (1994), identified by using the two SPME fibers. It is quite clear from that table that neither fiber was able to extract all the compounds from the oil's headspace. PDMS, a non-polar fiber, showed low sensitivity to polar compounds of virgin olive oil. On the other hand, the bi-polar PDMS/DVB fiber gave the overall best sensitivity for all classes of compounds in virgin olive oil. In that aspect the two SPME fibers can be used for a rough qualitative evaluation of the olive oil's flavor with the obvious limitations seen in Table 3. According to Roberts et al. (2000) the development of more sensitive and more polar fibers would be considered as a better way to extract headspace volatiles by the SPME technique. For samples containing a complex mixture of compounds, molecular competition for the active sites of the fiber during extraction is unavoidable, which influence the results (Roberts et al., 2000). This competition is driven by the different bonding energy associated to specific fiber–compound interaction, which may exclude compounds of interest. Another important phenomenon is the swelling of the fiber. This can greatly influence the retention of compounds as well as affect the catharsis of the fiber during desorption in the GC port. As a result of the above, we have recorded very low, or no amounts at all, of pentane, *n*-decane, *n*-heptane, and *n*-hexane ad-

Table 3
Characteristic virgin olive oil flavor volatile compounds isolated and identified using SPME with PDMS/DVB and PDMS fibers

Flavor volatile compounds	Sensory properties	Flavor volatile compounds	Sensory properties
Methyl acetate		Hexanal • Δ	Fatty, powerful, oily grassy
Octene	Solvent-like	Nonanal • Δ	Fatty, waxy, painty, citrus
Ethyl acetate • Δ	Sweet, aromatic	2-Heptenal • Δ	Oxidized, tallowy, pungent
2-Butanone Δ	Fragrant, pleasant	2-Decenal • Δ	Painty, fishy, fatty
3-Methyl butanal • Δ	Sweet, fruity	Pentanal • Δ	Woody, bitter, oily
1,3-Hexadien-5-yne Δ		2,4-Heptadienal Δ	Fatty, rancid, cinnamon
Ethyl furan	Sweet	Undecanal Δ	Fatty, tallow
Ethyl propanoate	Sweet, strawberry, apple	Heptanal • Δ	Oily, fatty, heavy, woody, penetrating, nutty
3-Pentanone Δ	Sweet	Octanal • Δ	Fatty, sharp, citrus
4-Methyl-pentan-2-one	Sweet	2-Nonenal • Δ	Penetrating, waxy, beany, rancid
Pent-1-en-3-one Δ	Sweet, strawberry	Decanal Δ	Penetrating, sweet, waxy, painty
2-methyl butenal Δ	Solvent-like	2,4-Decadienal • Δ	Pawerful, fatty, citrus
3-methyl butenol • Δ	Sweet apple	2-Hexenal Δ	Sweet, fragrant, almond, green, leafy
Methyl benzene	Glue, solvent-like	2-Octenal	Brown beans, herbaceous, spicy
Butyl acetate	Green, pungent, sweet	2-Undecenal • Δ	Fresh, fruity, orange peel
Hexyl acetate Δ	Sweet, fruity	2-Methyl butyl propanoate	Aromatic, ketone
Octan-2-one Δ	Moldy	2-Methyl-1-propanol • Δ	Ethyl acetate-like
3-(4-methyl-3-pentenyl) furan	Paint-like strong	(E)-2-pentenal	Green, apple
3-Hexenyl acetate Δ	Green banana, green leaves	(Z)-2-pentenal	Green, pleasant
(Z)-2-penten-1-ol • Δ	Banana	Ethyl benzene	Strong
6-Methyl-5-hepten-2-one	Fruity	(E)-3-hexenal • Δ	Artichoke, green
Nonan-2-one	Fruity	(Z)-3-hexenal	Green, green leaves
Hexan-1-ol • Δ	Fruity, aromatic, soft	1-Penten-3-ol • Δ	Wet earth
(E)-3-hexenol • Δ		Heptan-2-one •	Fruity
2,4-Hexadienal		(E)-2-Hexenal • Δ	Bitter almonds
(E)-2-hexen-1-ol • Δ	Green, grassy	(Z)-2-hexenal • Δ	Fruity, almonds
Acetic acid • Δ		2-Methyl butan-1-ol • Δ	Fish oil
Methyl decanoate	Fresh	3-Methyl-2-butenyl acetate	Putty-like, unpleasant
Tridecane Δ		Dodecane • Δ	
(Z)-3-hexen-1-ol Δ	Banana	Pentan-1-ol Δ	Pungent
3-Methyl butanol • Δ		Ethenyl benzene	

• indicates the compounds isolated by SPME-PDMS fiber.
Δ indicates the compounds isolated by SPME-PDMS/DVB fiber in this work.
Characteristic olive oil compounds by Kiritsakis (1998), Aparicio and Morales (1998) and Morales et al. (1994).

sorbed by the polar PDMS/DVB fibers, although they have been reported to be present in substantial amount in olive oil (Aparicio & Morales, 1998) and high oleic oxidized oils (Warner, Evans, List, Boundy, & Kwolek, 1974).

In order to obtain higher amounts of volatiles and the highest number of individual volatile compounds by the SPME static headspace analysis technique, the following variables were tested: equilibrium time before sampling, amount oil/headspace ratio, and sampling time. Lower oil/headspace ratio (3.5 g /12 ml vessels), higher sampling time (30–40 min), and lower equilibrium time (2 h) for the PVDS-DVB fiber were selected as the optimum conditions for all SPME extractions (Fig. 4).

Although all the sampling combinations were also performed for the 7 and 10.5 g, due to extended data available, Fig. 5 shows only the results for a few characteristic sampling times and equilibrium times that gave the higher separation efficiency when SPME with PDMS-DVB was used. Long equilibrium times led to the retention of fewer compounds, and those retained were predominantly higher molecular weight compounds. It appears that with time, low molecular weight compounds were facing a stronger competition by the larger molecules that were available at long equilibrium times. That probably led to a competitive and dynamic re-arrangement and re-occupation process of the available active sites of the fiber especially under long (40 min) sampling times. That phenomenon was not as apparent when flavor compounds were extracted from 7 to 10.5 g of oil. Besides the potential risk of compounds adsorbed by the glass wall after such long times, it might also be possible that for the small headspace available in these cases, the efficiency and accuracy of the sampling was decreased due to higher influence of the sampling parameters based on competitive conditions among volatile compounds for the limited space. Further studies are needed to establish the thermodynamic basis of this process.

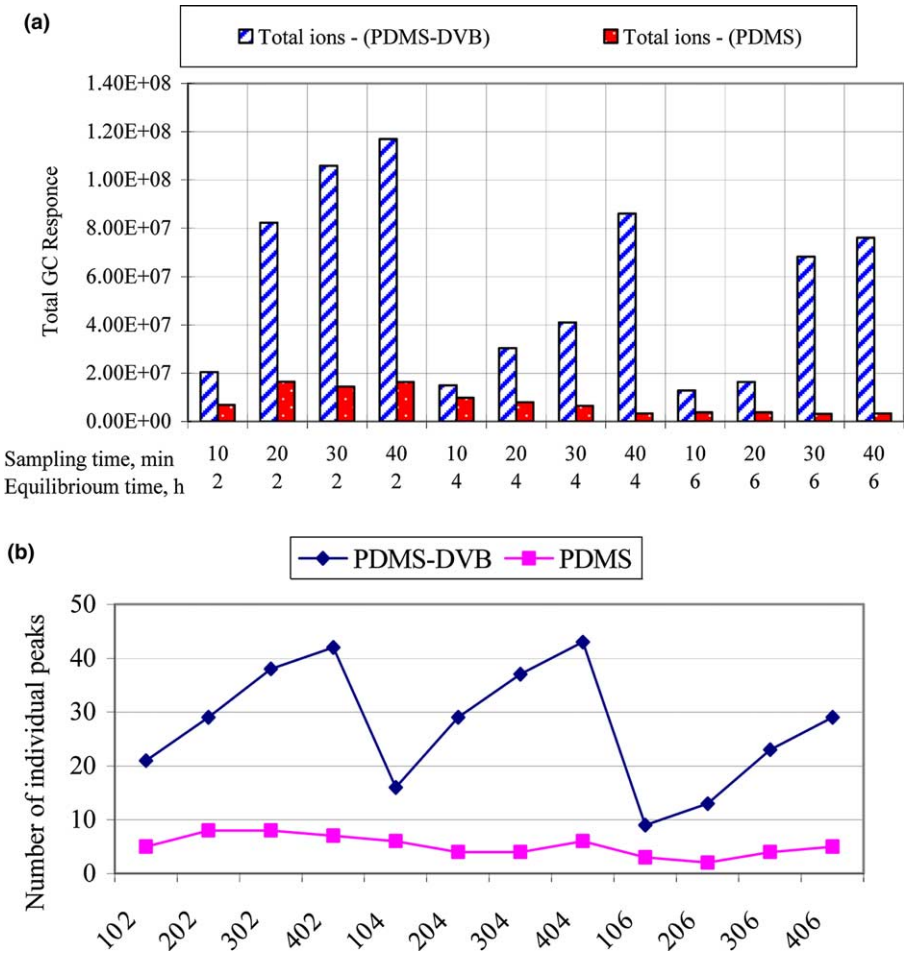

Fig. 4. Total gas chromatography response from 3.5 g of olive oil placed in 12.5 ml glass vials, equilibrated for headspace generation at 23 °C for 2, 4 and 6 h. Flavor volatile compounds were extracted from the headspace using solid phase micro-extraction technique with PDVB-DVB and PDVB fibers exposed for 10, 20, 30 and 40 min. (a) Total gas chromatography response and (b) total number of peaks isolated.

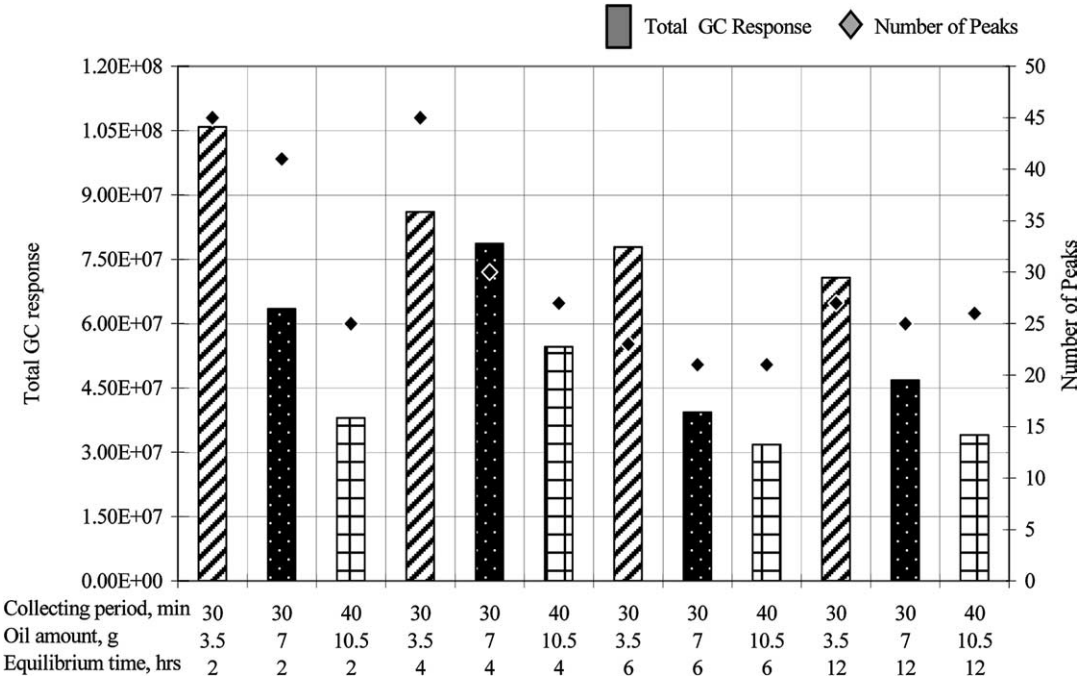

Fig. 5. Sampling of virgin olive oil flavor compounds with SPME with PDMS-DVB. Summary of the best collecting periods and oil amounts, for different equilibrium periods. Left axis: total GC response; right axis: number of peaks.

4. Discussion

This study showed that both DHS-TD/GC/MS and SPME-GC/TOFMS techniques were very efficient and accurate for isolation and identification of virgin olive volatile compounds when applied at the proper operating conditions, trapping materials, and temperature.

For DHS-TD/GC/MS a careful evaluation of the variables should be considered. These variables include the He volume/oil mass ratio, trapping material, sampling time, temperature, and desorption conditions. The values of the He volume/oil mass ratio appeared to have a significant effect on the isolated compounds in terms of their amounts present. Furthermore, analytical conditions should be selected according to whether the interest is to isolate low or higher molecular volatile compounds, or a combination of both. Recognizing the effect of the trapping material we have to comment on the necessity of evaluating other trapping materials.

The applications described herein demonstrate how the analysis of olive oil flavor compounds can be easily accelerated by a factor of 5–10 simply by using smaller column dimensions and higher helium flow rates. Thus, TOFMS permits identification of compounds in about 7–8 min compared to the approximately 1 h required by the conventional purge and trap-GC analysis. The analyses performed on the GC-TOF-MS-system demonstrated high sensitivity and also high selectivity due to the high quality of mass spectra obtained. In this study, the SPME-GC/TOFMS technique appears to be faster and simpler than DHS-TD/GC/MS but the latter provided a higher efficiency. The advantages of GC-TOF-MS make it a promising analytical technique for olive oil flavor analysis. SPME might be more suitable for trace analysis where headspace concentrations of volatiles are relatively low. At relatively high volatile concentrations however, molecular competition effects may lower its sensitivity. The performance of more polar fibers for extracting accurately and easily a greater number of volatiles present in olive oil should be further evaluated. In a manuscript under preparation we describe the application of these techniques to the analysis of thermo-oxidized olive oil.

Acknowledgements

This paper is dedicated to the memory of the late Dr. Ruben J. Hernandez. The Michigan State University (MSU) Agriculture Experimental Station is highly acknowledged for the provided financial support. Many thanks to Dr. R.M. Beaudry (Department of Horticulture, MSU) for helping with SPME-TOF/MS, and D. Gage and J. Watson (Department of Biochemistry, MSU) for helping with Mass Spectrometry.

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