Supporting Information

Capturing plant metabolome with direct-immersion in vivo solid phase microextraction of plant tissues

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Abstract:

Supporting Information contains Experimental Section and provides details on analytical reagents and supplies, sample collection, sample preparation for *ex vivo* SPME and additional information associated with *in vivo* DI-SPME sampling procedure. Details on conditions for acquisition and processing of metabolomics data on GCxGC-ToFMS are also given. Results and Discussion section contains relevant figures and tables, as noted in the text.

Experimental Section

Analytical reagents and supplies

Acetone and methanol of HPLC grade were purchased from Caledon Laboratories (Georgetown, ON, Canada). For development of system precision checks and compilation of GCxGC, mass spectral and linear temperature-programmed retention index (RI) databases for metabolites commonly detected in foods, spiked water samples were analyzed by HS-SPME. Metabolite standards[1] for system precision and database compilation were purchased from Sigma–Aldrich (Oakville, ON, Canada). The purity of all standards was greater than 97%, with the exception of heptanal, nonanal, citral isomers, farnesol isomers, dodecanal, tridecanal, and linalool, all of which had purity > 95%. 10 mL amber screw cap vials and SPME holder were purchased from Supelco (Oakville, ON, Canada).

Samples and sample preparation for ex vivo SPME

'Honeycrisp' apples (with a diameter of approximately 6-7 cm) were harvested after on-site sampling from a mature commercial orchard in Simcoe (Norfolk County, ON, Canada). Immediately after harvesting, fruit were immersed in liquid nitrogen and subsequently stored in dry ice (-70 ^{o}C) during transportation to the laboratory. In the laboratory, individual fruit were rinsed with distilled water and dried with Kim Wipe. Frozen fruit were sliced in random positions from all possible sides of the fruit cortex. One hundred grams of frozen apple tissue was homogenized for 1.5 min with 250 mL of saturated sodium chloride solution. An additional 250 mL aliquot of nanopure water was added to the homogenate, and the apple slurry was homogenized for an additional 1 min. The final homogenate was transferred into 20 mL vials, protected from light and stored in freezer at – 30 ^{o}C. At the time of analysis, vials containing homogenate were thawed for 20 min individually in a temperature-controlled water bath maintained at 30 ^{o}C. 10 mL and 3 mL portions of thawed homogenate were transferred into 10 mL screw-cap amber vials for DI-SPME and HS-SPME, respectively, followed by 5 min incubation and 60 min extraction at 30 ^{o}C and 500 rpm. After DI-SPME extraction, a brief

immersion of SPME extraction phase in 10 mL of nanopure water prior to desorption was carried out to remove non-volatile interferences from the coating surface.

Retention indices in the first dimension were determined by HS-SPME extraction of aqueous samples spiked with 52 metabolites belonging to various chemical groups, including *n*-alkanes (C_8-C_{19}), aldehydes, 2-ketones, ethyl esters, monoterpenes (hydrocarbons, ketones, aldehydes, oxides and alcohols), sesquiterpenes (hydrocarbons, alcohols), 1-alcohols, and 2-alcohols, using same extraction conditions as for HS-SPME and DI-SPME analyses of apple samples (preparation of spiked aqueous standards was carried out as per procedure in reference 1). This mixture was also analyzed on a regular basis in order to provide a quick measure of stability of instrumental response and to ensure no degradation of modulation efficiency and column resolving power occurred during the analysis of long sample sequences. In addition, homogenate portions from individual apples were combined and homogenized to form a QC mixture representative of apple metabolome. 3 mL portions of this mixture were transferred to 10 mL vials and also analyzed by HS-SPME using the same extraction conditions as described above for HS-SPME and DI-SPME analyses of apple samples.

In vivo DI-SPME procedure and sampling set-up

The purpose of *in vivo* sampling in the 2009 harvesting season (in-field temperature 16 ^{o}C) was determination of intra-fruit repeatability. This was conducted by employing triplicate SPME extractions per apple using the design in which fibre coatings were penetrated into the apple cortex from directions that were perpendicular with respect to the fruit stem. In order to allow metabolome sampling from three distinct sides of apple fruit, the coatings were kept as far as possible from each other (sampling design 1).

On the other hand, determination of analytical precision and statistical evaluation of acquired data for fruit at two different maturity stages were conducted during sampling in 2010 (outside temperature ranging from 24 ^{o}C at the beginning of sampling to 21 ^{o}C at the end of experiment). Five apples of earlier maturity index (HC-O apples, codes 1-5) (7 starch index (based on Cornell starch chart, 1-8 scale (8=no starch)), 40-60 ppm internal ethylene concentration, 14-15 lb firmness, average 12.4% soluble solids and 570 mg malic acid per 100 mL juice, 70-80% red blush with yellow background color) and 5 apples of later harvest maturity

(HC-L apples, codes 1-5) (8 starch index (based on Cornell starch chart, 1-8 scale (8=no starch)), 20-40 ppm internal ethylene concentration, 13-14 lb firmness, average 12.9% soluble solids and 520 mg malic acid per 100 mL juice, 80-90% red blush with yellow-green background color) were sampled with triplicate analysis per apple using sampling positions 1.5 cm apart from each other (sampling design 2).

The objective of the sampling conducted in 2011 (temperature on the site of sampling 18 ^{o}C) was to conduct a comparison of metabolite coverage obtained using DVB/CAR/PDMS and PDMS overcoated-DVB/CAR/PDMS fibre coatings so as to determine whether more effective clean-up of the coating surface after DI-SPME minimized thermal decomposition reactions of non-volatile and thermally labile components.

Conditions for acquisition and processing of metabolomics data on GCxGC-ToFMS

GC inlet was equipped with a 0.75 mm ID narrow-bore liner from Supelco (Oakville, ON, Canada) and a high-pressure Merlin Microseal septumless injection system from Merlin Instrument Co. (Half Moon Bay, CA, USA). Desorption was carried out at 270 ^{o}C after careful optimization of desorption efficiency based on the results presented in a previous study.[1] Helium was used as carrier gas with a flow rate of 2.0 mL/min. The primary dimension oven temperature programming was set to 40 ^{o}C (5 min hold), followed by 5 ^{o}C/min rate to 235 ^{o}C (10 min). The secondary oven programming was equivalent except for the 20 ^{o}C temperature offset above the primary oven temperature. The modulation parameters included a modulator temperature offset of 25 ^{o}C, and a 3.5 s modulation period (0.7 s hot pulse time, 1.05 s cool time). The acquisition rate was 200 spectra/sec.

For the determination of metabolome coverage, the GCxGC conditions consisted of Rxi-5SilMS and BP 20 (1.11 m x 0.10 mm ID x 0.10 μm) for the first and second dimension columns, respectively. Helium was used as carrier gas with a flow rate of 1.5 mL/min. The primary dimension oven temperature programming was set to 40 ^{o}C (5 min hold), followed by 5 ^{o}C/min rate to 250 ^{o}C (10 min hold). The secondary oven programming was equivalent except for a 10 ^{o}C temperature offset. The modulation parameters consisted of a modulator temperature offset of 30 ^{o}C and a 4 s modulation period (0.8 s hot pulse time, 1.20 s cool time). An acquisition rate of 250 spectra/s was employed.

For all studies, the transfer line and ion source temperatures were set to 240 and 220 ^{o}C, respectively. The mass spectrometer was operated in electron ionization (EI) mode with a mass acquisition range of 33-550 *u*. Data acquisition and processing were performed with ChromaTOF (version 4.24) software. National Institute of Standards and Technology (NIST, version 2.05), Terpene, and Wiley 8 mass spectral databases were available for library searching.

Data processing consisted of several steps; first, following processing of raw data, mass spectral deconvolution and second dimension peak combination, peak table entries meeting certain mass spectral similarity threshold (not less than 700) were preserved and further manipulated. For statistical analysis on discrimination between metabolic profiles corresponding to fruit with different maturity levels, the sample for which the highest number of one-dimensional peak entries was obtained was used as a reference for data reduction and compilation of a reliable data matrix. Considerable emphasis was placed on manual picking of high quality metabolites and elimination of false outlying features. Manual processing was carefully initiated in order to filter out: i) column bleed peaks, fibre bleed peaks, and blank peaks; ii) peaks for which separation efficiency and modulator effectiveness were not optimum, resulting in a multitude of outlying deconvolutions; iii) peaks with overloaded and tailing peak profiles resulted by non-linear chromatography, second dimension column and modulator overloading, and analyte-stationary phase incompatibility; iv) metabolite entries for which one-dimensional peaks were represented by streaking peak profiles and iso-volatility curves. Replicate mass spectral assignment entries were preserved provided that the criterion of unique elution on a two-dimensional separation plane was met. In total, 225 true high-quality metabolites were submitted to automated 'compare-to-reference' ChromaTOF software alignment procedure. Manual inspections of the quality of data processing were carried out for each single metabolite. Due to the complexity of *in vivo* extracts, corrections accounting for unique mass misassignment, metabolite misalignment, incorrect second dimension peak combination into respective one-dimensional peak entries, and incorrect second dimension peak integration were carried out in 50% of cases. Annotation of metabolite identity was performed on the basis of retention time and mass spectral comparison with reference standards, retention index comparison, and GCxGC molecular structure-retention relationships.

Figure S-1. SPME sampling design approach 1 from 2009 sampling season for evaluation of intra-fruit repeatability

Results and Discussion

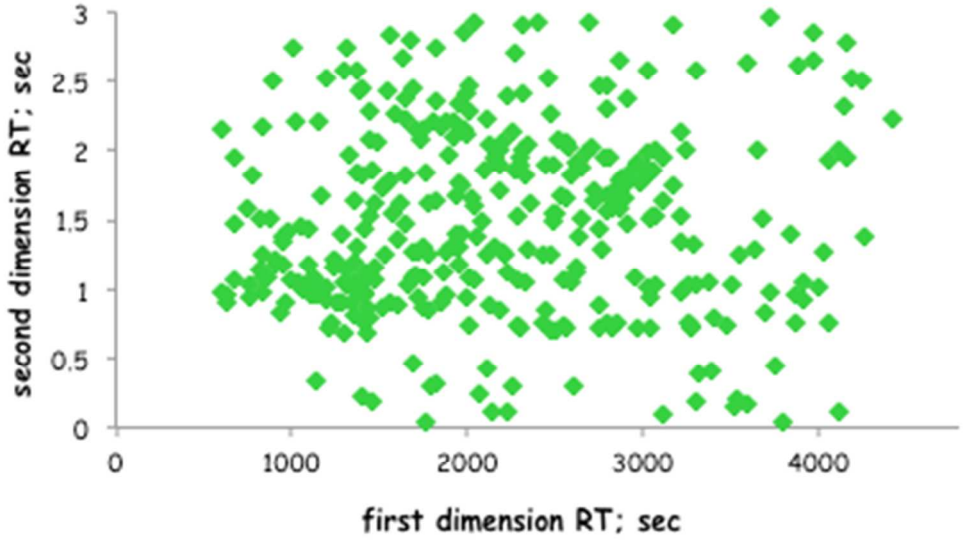


Figure S-2. GCxGC peak apex plot with retention time coordinates of 357 true metabolites included in global evaluation of analytical precision

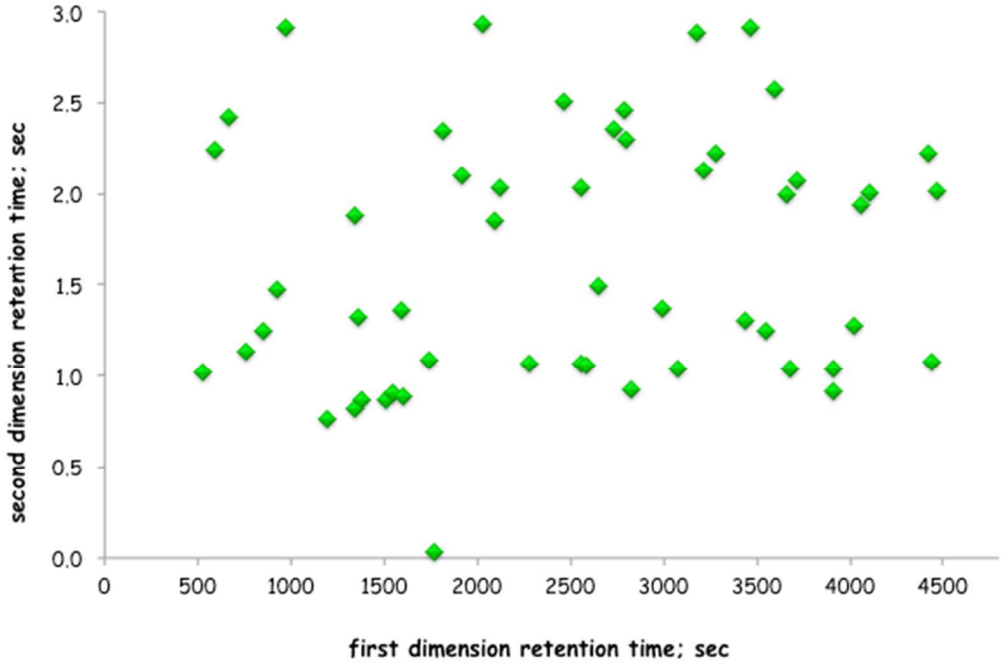


Figure S-3. The peak apex plot of GCxGC retention times corresponding to tentatively identified metabolites included in evaluation of intra-fruit repeatability of *in vivo* DI-SPME – GCxGC-ToFMS

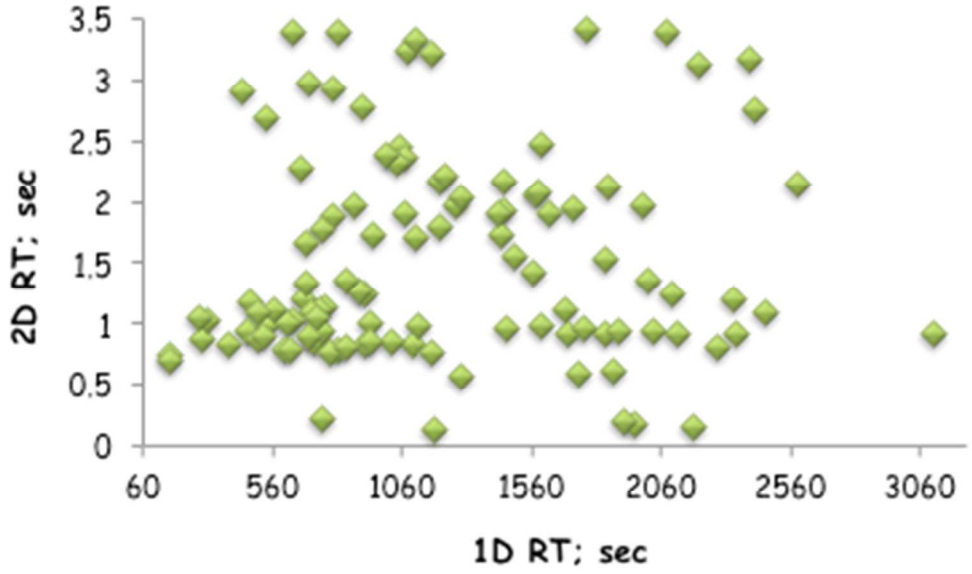


Figure S-4. Peak apex plot illustrating elution times of metabolites included in global evaluation of intra- and inter-fruit repeatability in September 2010 season (sampling design 2)

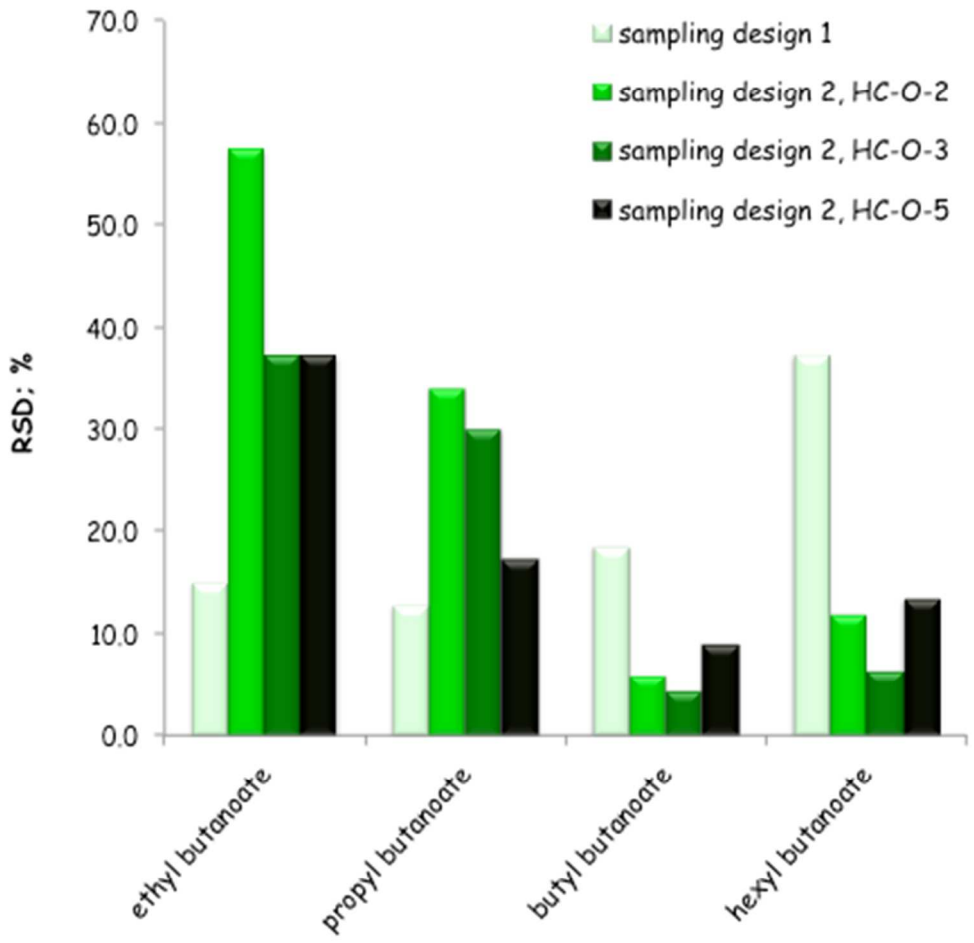
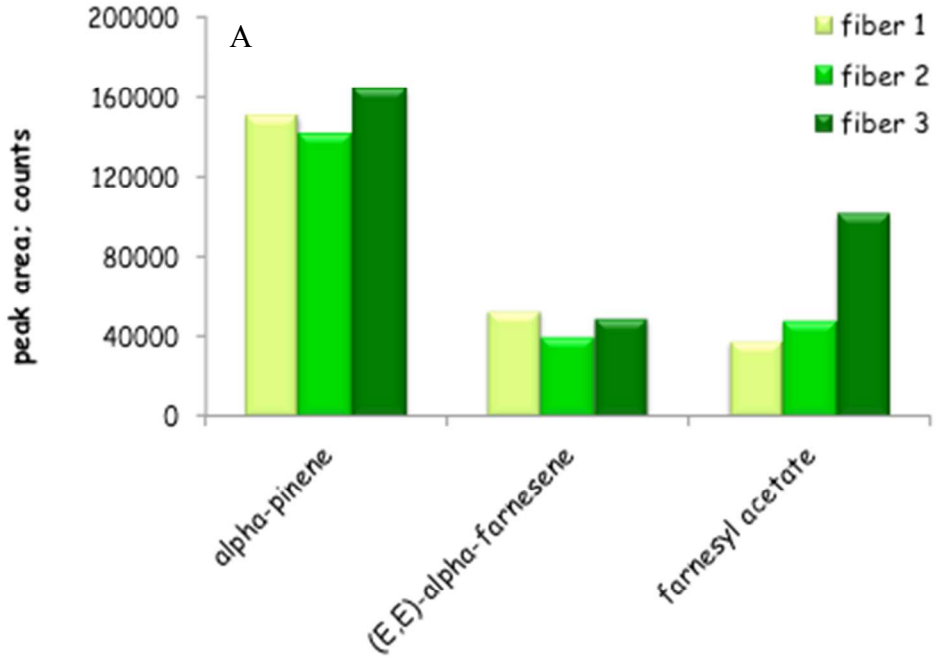


Figure S-5. Comparison of analytical precision corresponding to two different *in vivo* sampling designs (sampling design 1 and 2 adopted in 2009 and 2010 seasons, respectively) for series-related compounds


A
peak area; counts
200000
160000
120000
80000
40000
0
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fiber 2
fiber 3
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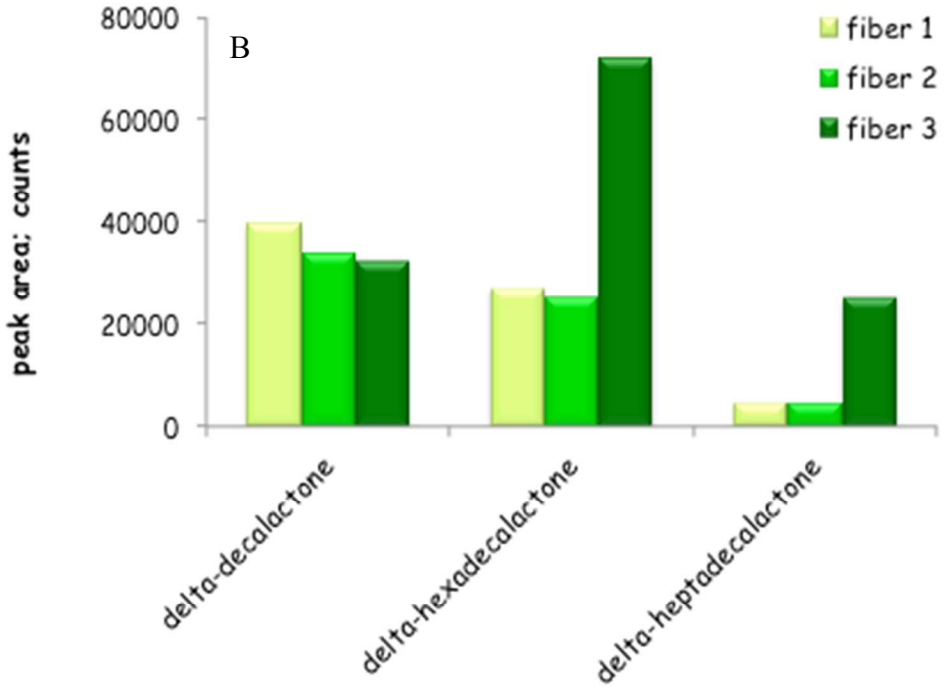
(E,E)-alpha-farnesene
farnesyl acetate


B
peak area; counts
80000
60000
40000
20000
0
fiber 1
fiber 2
fiber 3
delta-decalactone
delta-hexadecalactone
delta-heptadecalactone

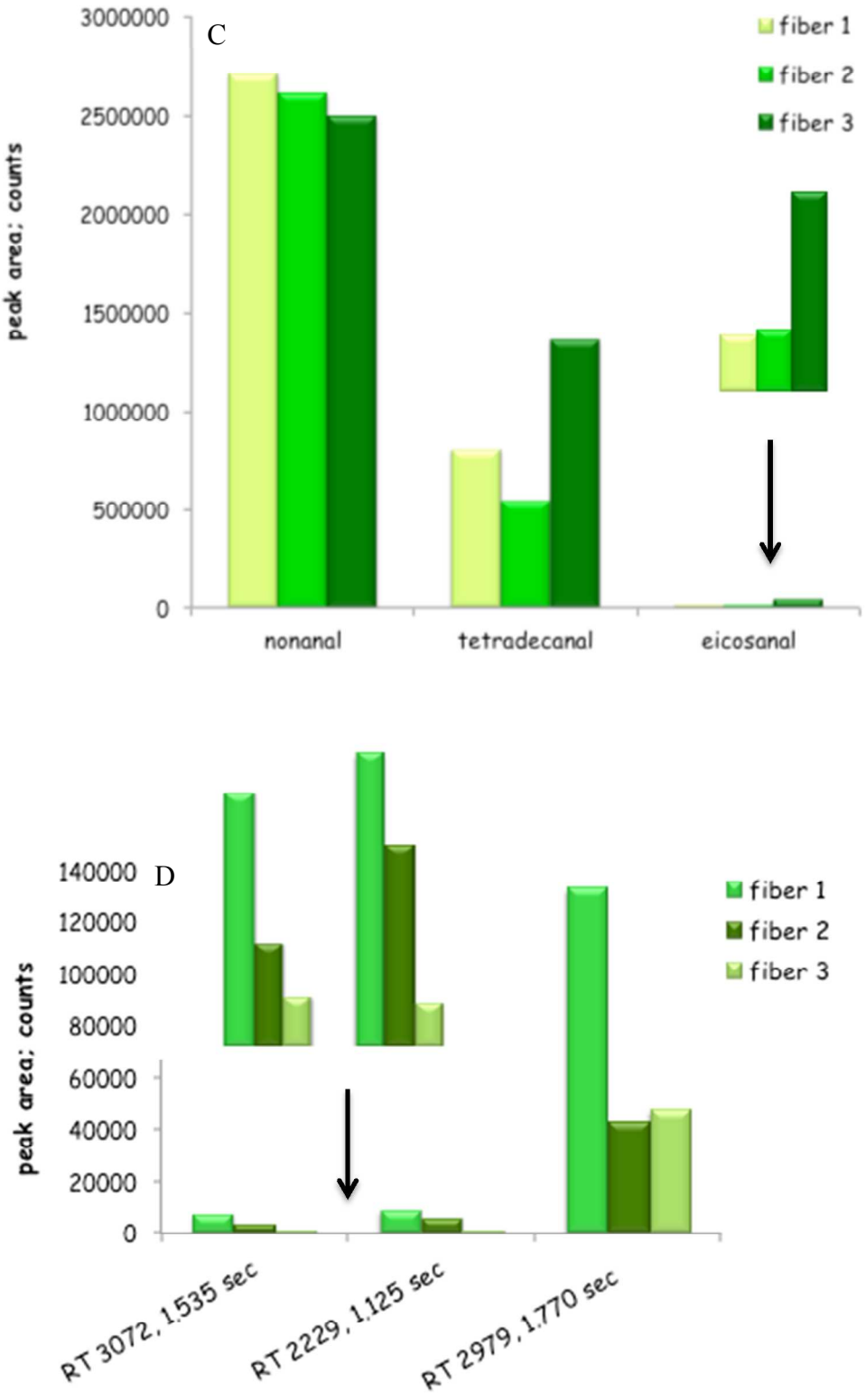
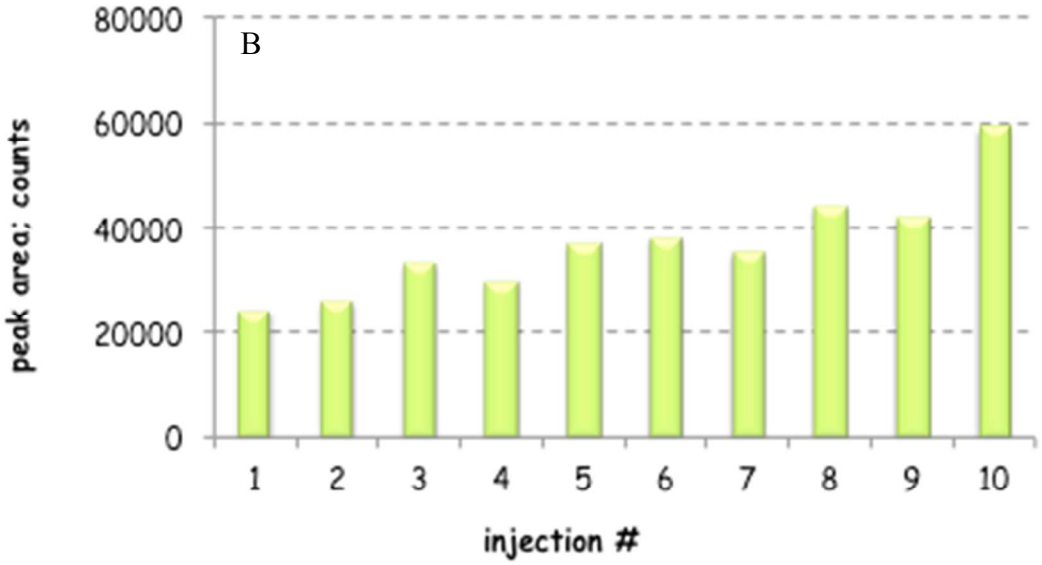
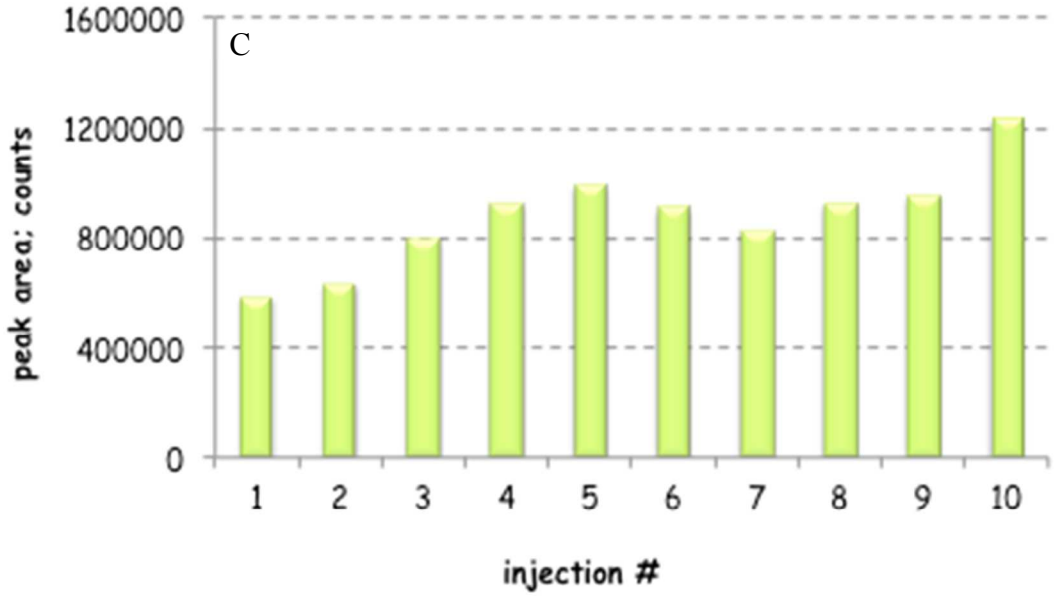
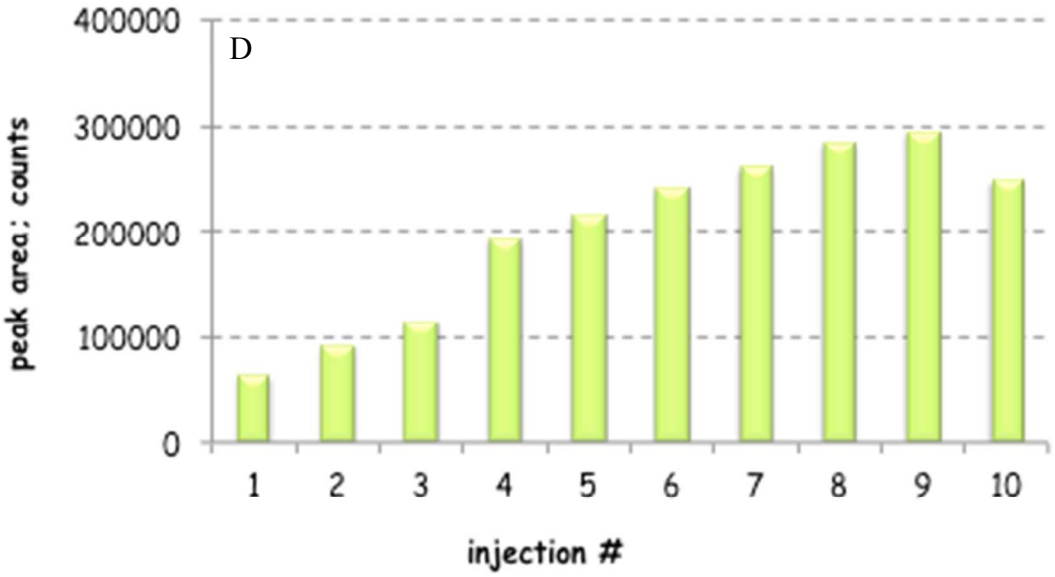


Figure S-6. Comparison of the extraction efficiencies of three fibre coatings employed in *in vivo* DI-SPME sampling for selected low and high boiling point compounds in homologous series of A –

terpenoids, B – delta-lactones and C – aldehydes and a selected high molecular weight metabolite which was not tentatively identified (plot D)




C
1600000
1200000
800000
400000
0
peak area; counts
1
2
3
4
5
6
7
8
9
10
injection #


D
400000
300000
200000
100000
0
peak area; counts
1
2
3
4
5
6
7
8
9
10
injection #

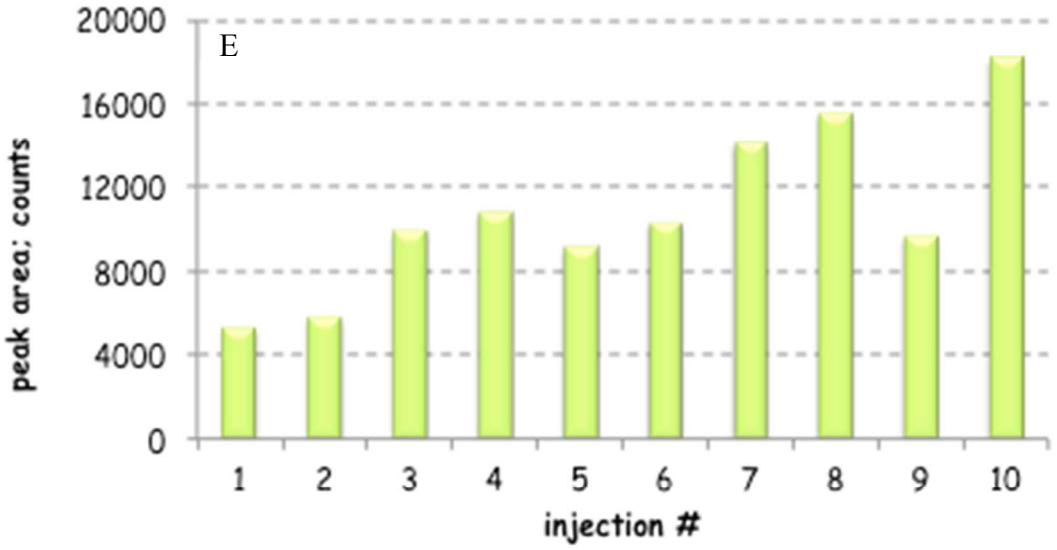
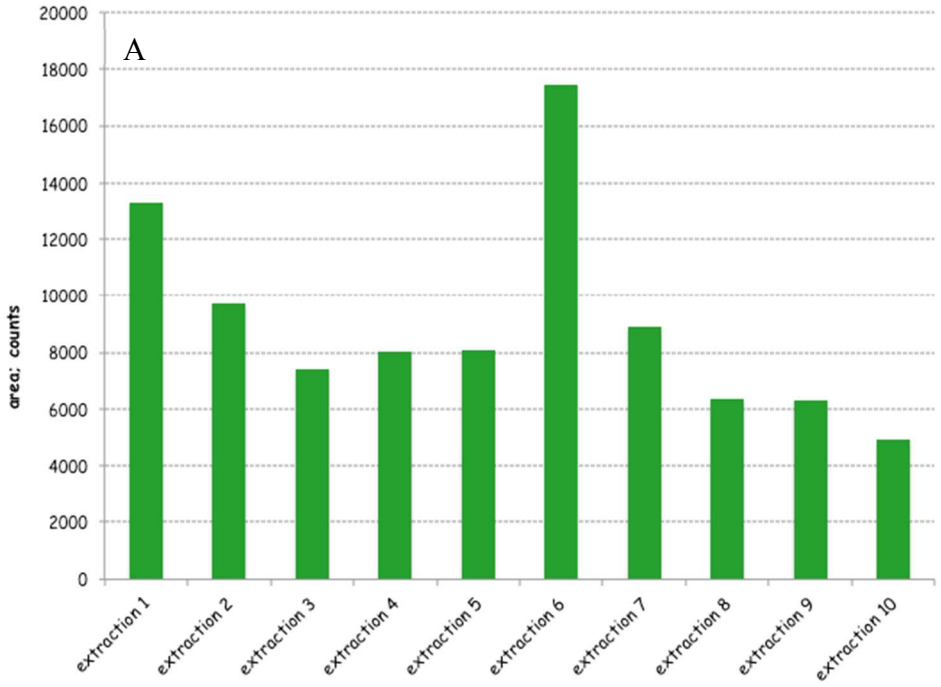


Figure S-7. Intra-fruit repeatability and stability of selected metabolites detected by HS-SPME. A - 6-methyl-5-hepten-2-one, B - *beta*-myrcene, C - (2E)-2-octenal, D - *trans*-*beta*-damascenone, and E - (*Z*,*Z*)-farnesol.



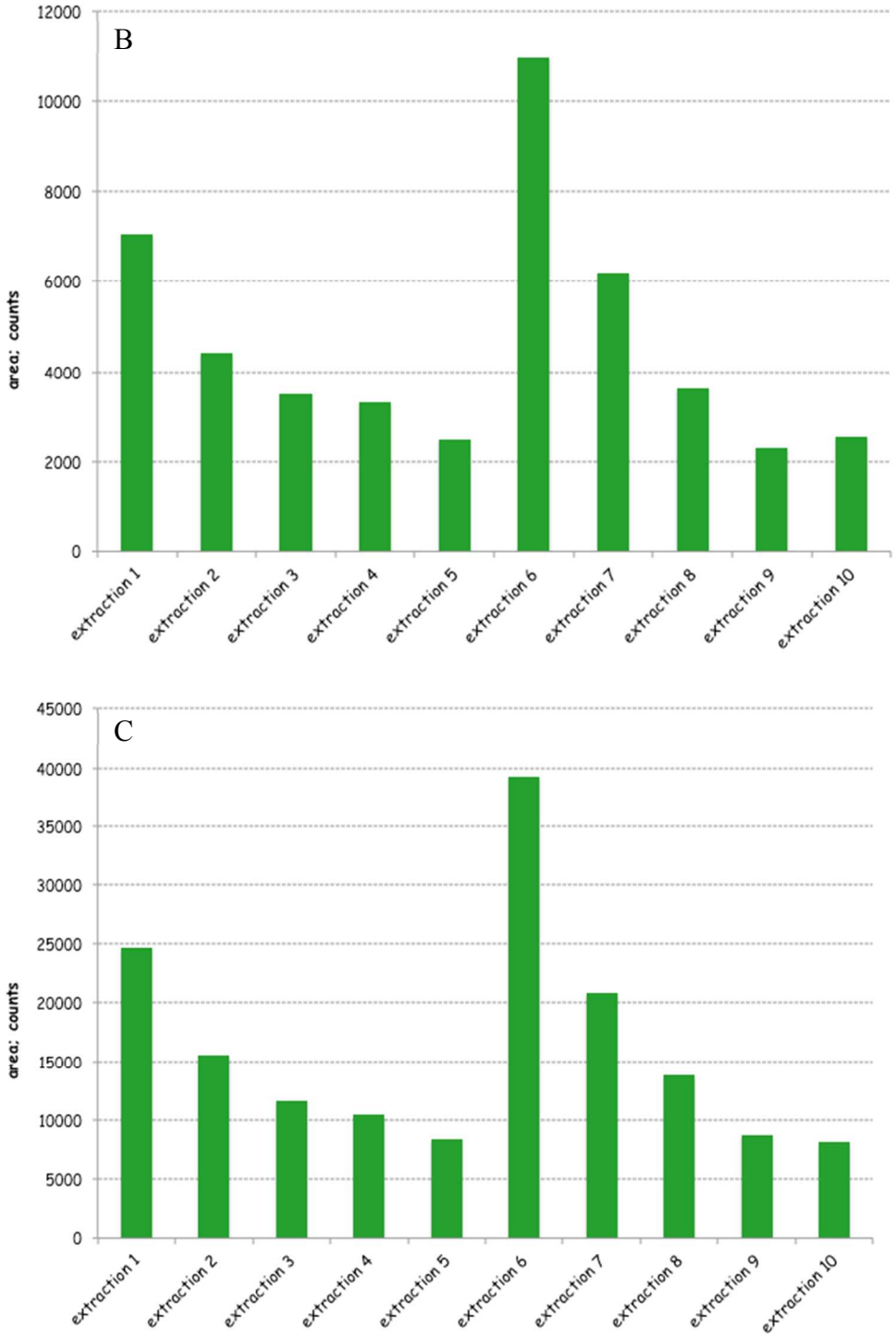


Figure S-8. Reproducibility obtained in HS-SPME extraction of hydrophobic metabolites (A – 1-tridecanol, B – 1-pentadecanol, C – 2-heptadecanone) when samples (same homogenate, different storage vials during freeze storage) are submitted to extraction immediately after sample preparation.

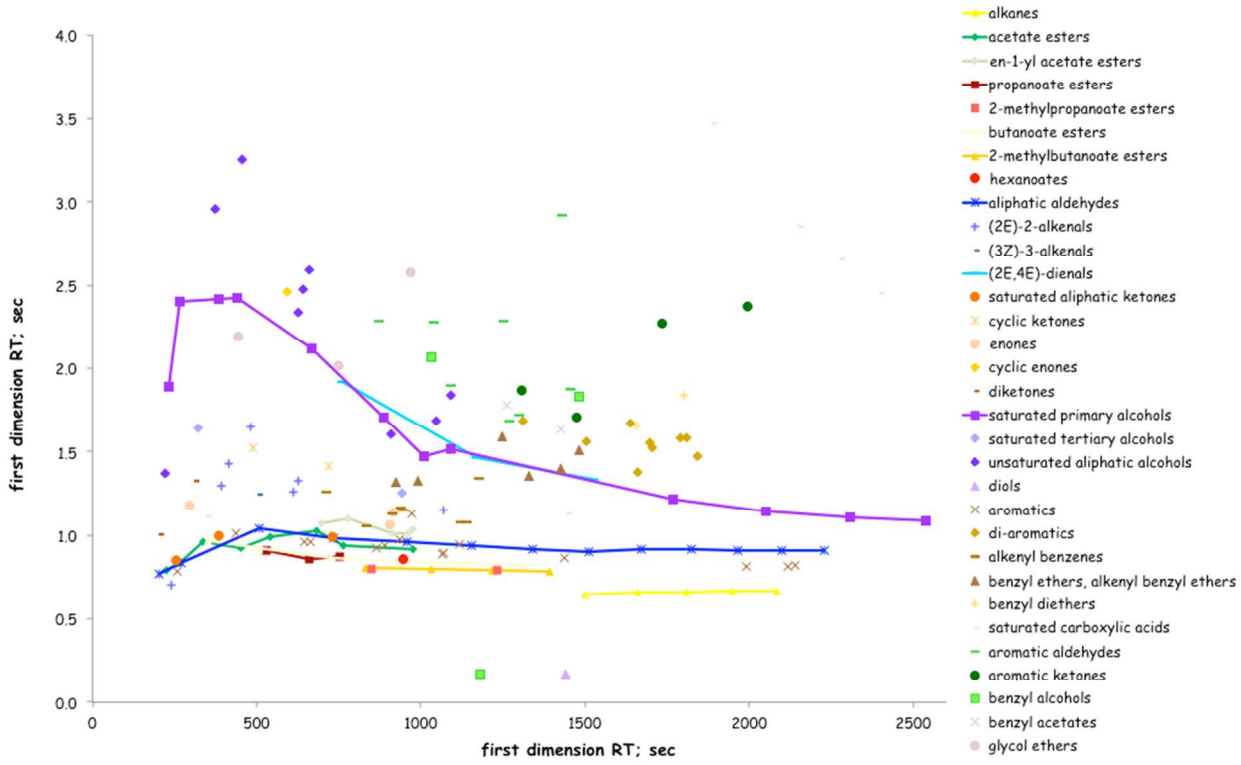


Figure S-9. Peak apex plot illustrating retention time coordinates of metabolites captured by *in vivo* SPME that were tentatively identified and grouped in respective chemical classes

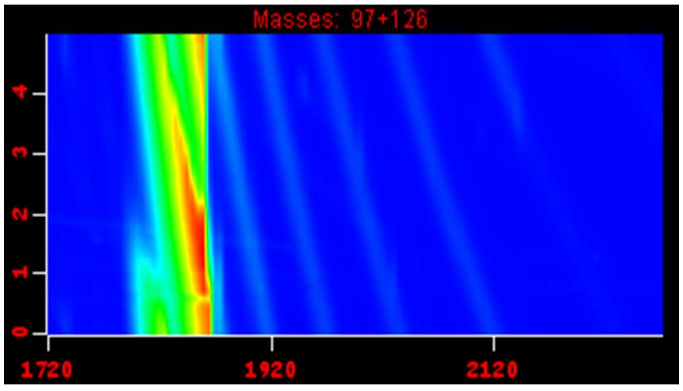


Figure S-10. GCxGC extracted ion chromatogram corresponding to elution window of 5-(hydroxymethyl)-2-furfural in *in vivo* metabolic profile obtained by DVB/CAR/PDMS fibre coating

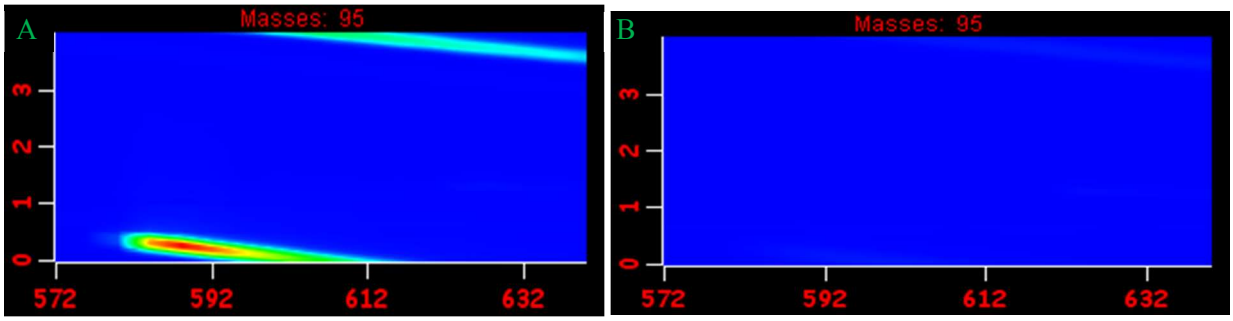


Figure S-11. GCxGC extracted ion chromatograms corresponding to elution windows of furfural in *in vivo* metabolic profiles obtained by A – DVB/CAR/PDMS and B – PDMS-overcoated DVB/CAR/PDMS fibre coatings

Table S-1. Tentatively identified metabolites and their retention data for the experiment associated with global evaluation of intra-fruit repeatability of *in vivo* DI-SPME – GCxGC-ToFMS

analyte name; *(synonym)*	^1t_R; s	^2t_R; s	RI_{exp}	RI_{lit}	RSD; %
acetic acid, propyl ester	525	1.020	718	na	**13.2**
2-methyl-1-butanol	588	2.245	741	731	**25.0**
1-pentanol	666	2.420	770	759	**28.4**
Hexanal	759	1.135	803	801	**13.2**
2-vinyl-5-methylfuran	852	1.250	831	na	**24.7**
2-hexenal	930	1.475	855	850	**20.8**
trans-2-hexenol	972	2.915	867	na	**19.1**
2,6,6-trimethylbicyclo[3.1.1]hept-2-ene, *(alpha-pinene)*	1194	0.760	934	933	**7.2**
6,6-dimethyl-2-methylene-bicyclo(3.1.1)heptane, *(beta-pinene)*	1344	0.825	979	978	**0.3**
1-octen-3-ol	1347	1.885	980	978	**11.7**
6-methyl-5-hepten-2-one	1362	1.320	985	986	**9.3**
2-methyl-6-methylene-2,7-octadiene, *(beta-myrcene)*	1380	0.865	990	991	**11.7**
1-methyl-4-(1-methylethenyl)cyclohexene, *(limonene)*	1509	0.870	1030	1030	**11.0**
butyl 2-methylbutanoate	1545	0.905	1042	na	**23.6**
2-octenal	1596	1.365	1058	1059	**3.7**
1-isopropyl-4-methyl-1,4-cyclohexadiene, *(gamma -terpinene)*	1602	0.890	1059	1058	**12.2**
Nonanal	1743	1.090	1104	1107	**4.1**
cis,cis-4,6-octadienol	1767	0.035	1112	na	**14.4**
butyl-3-hydroxybutanoate	1818	2.345	1129	na	**16.8**
4-ethylbenzaldehyde	1920	2.100	1164	1181	**9.0**
(2E)-3-phenyl-2-propenal, *(trans-cinnamaldehyde)*	2034	2.930	1202	na	**14.9**
4-isopropylbenzaldehyde, *(cumaldehyde)*	2091	1.855	1223	na	**6.6**
1-benzofuran-2(3H)-one, *(2-coumaranone)*	2124	2.030	1234	na	**15.6**
2-undecanone	2283	1.070	1291	1294	**19.8**
5-pentyldihydro-2(3H)-furanone, *(gamma-nonalactone)*	2469	2.505	1361	1362	**13.4**

2-dodecanone	2556	1.065	1394	1393	**23.4**
1,2-dimethoxy-4-(2-propenyl)benzene, *(methyl eugenol)*	2562	2.030	1397	1403	**21.3**
Dodecanal	2589	1.060	1407	1410	**28.9**
1,3-diacetylbenzene	2646	1.495	1430	na	**4.3**
5-hexyldihydro-2(3H)-furanone, *(gamma-decalactone)*	2730	2.355	1465	1469	**12.5**
6-pentyltetrahydro-2H-pyran-2-one, *(delta-decalactone)*	2793	2.460	1490	1494	**10.7**
1-(4-methoxyphenyl)-3-butanone, *(rasberry ketone methyl ether)*	2799	2.295	1493	na	**10.6**
1,3,6,10-dodecatetraene, 3,7,11-trimethyl-, (3E,6E)-, *(farnesene <(E,E)-, alpha->)*	2826	0.925	1504	1504	**13.8**
1-tridecanol	2991	1.370	1574	1580	**14.4**
Tetradecanal	3078	1.040	1612	1614	**46.4**
phenyl benzoate	3180	2.890	1659	na	**25.4**
dihydro-5-octyl-2(3H)-furanone, *(gamma-dodecalactone)*	3219	2.125	1677	1681	**10.7**
6-heptyltetrahydro-2H-pyran-2-one, *(delta-dodecalactone)*	3279	2.220	1704	1708	**16.3**
1-pentadecanol	3435	1.305	1779	1784	**14.7**
valeric acid, 2-pentadecyl ester	3462	2.915	1791	na	**34.8**
2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, acetate, *(farnesyl acetate)*	3546	1.245	1833	1832	**55.0**
4-(1-methyl-1-phenylethyl)phenol, *(4-cumylphenol)*	3597	2.575	1858	na	**6.6**
5-decyldihydro-2(3H)-furanone, *(gamma-tetradecalactone)*	3657	2.000	1888	na	**22.6**
2-heptadecanone	3681	1.040	1900	1915	**43.0**
6-nonyltetrahydro-2H-pyran-2-one, *(delta-tetradecalactone)*	3717	2.070	1919	1920	**31.9**
Octadecanal	3909	1.040	2014	2024	**66.4**
1-methylethyl hexadecanoate, *(isopropyl palmitate)*	3912	0.915	2016	na	**32.8**
1-octadecanol	4023	1.275	2060	na	**60.7**
5-undecyldihydro-2(3H)-furanone, *(gamma-pentadecalactone)*	4056	1.935	2073	na	**72.1**
6-undecyltetrahydro-2H-pyran-2-one, *(delta-hexadecalactone)*	4110	2.010	2095	na	**63.7**
2-ethylhexyl-4-methoxycinnamate	4422	2.220	na	na	**48.2**
Eicosanal	4440	1.080	na	na	**77.5**
6-dodecyltetrahydro-2H-pyran-2-one, *(delta-heptadecalactone)*	4470	2.015	na	na	**102.3**

RI_{exp} – experimental linear temperature-programmed retention index
RI_{lit} – literature linear temperature-programmed retention index
na – not available

Table S-2. Intra-fruit repeatability obtained for selected high molecular weight metabolites using different approaches of sampling and sample preparation

	HS-SPME, fresh sample preparation, 10 vials	HS-SPME, vial 1 (*n*=3), vial 2 (*n*=3), vial 3 (*n*=4)	*in vivo* SPME
analyte name	RSD; % (*n*=10)	RSD; % (*n*=10)	RSD; % (*n*=3)
1-tridecanol	41.4	69.8	14.4
1-pentadecanol	58.7	38.4	14.7
2-heptadecanone	60.6	63.1	43.0

Table S-3. One-way ANOVA treatment of *in vivo* SPME extracted responses for butyl propanoate, butyl butanoate, ethyl hexanoate, butyl 2-methylbutanoate and estragole obtained for HC-O apple group (lower harvest maturity) and HC-L apple group (higher harvest maturity).

analyte name	Butyl propanoate	Butyl butanoate	Ethyl hexanoate	Butyl 2-methylbutanoate	Estragole
^1t_R; s	521.5	714	721	808.5	1106
^2t_R; s	0.905	0.860	0.890	0.785	1.720
interfruit RSD (HC-O, n=12); %	40.9	34.4	73.1	46.5	12.9
interfruit RSD (HC-L, n=12); %	38.3	30.2	52.1	24.6	26.7
F	7.4	10.9	14.4	27.6	10.4
F_{crit}	6.0	6.0	6.0	6.0	6.0
P	3.5	1.7	0.9	0.2	1.8

Table S-4. Metabolites extracted by *in vivo* DI-SPME that were identified on the basis of retention time and mass spectral comparison with reference standards, retention index comparison, and GCxGC molecular structure-retention relationships.

analyte name	cas #	^1t_R; sec	^2t_R; sec	RI_{exp}	RI_{lit}	unique mass	SIM
Butanal		204	0.768	600	596	72	839
3-Buten-2-one	78-94-4	204	0.920	600	606	55	936
2,3-Butanedione	431-03-8	204	1.004	600	592	86	968
2-Methylfuran	534-22-5	212	0.760	608	608	82	934
Methylbutenol	60766-00-9	220	1.368	616	611	71	898
Ethyl Acetate	141-78-6	224	0.792	620	614	61	935
Trichloromethane	67-66-3	228	1.140	624	629	83	913
2-Methyl-1-propanol	78-83-1	232	1.888	628	626	33	848
2-Butenal	4170-30-3	240	0.704	636	640	70	808
2-Pentanone	107-87-9	256	0.852	652	682	58	768
Benzene	71-43-2	260	0.788	656	658	78	954
2-Propyl acetate	108-21-4	264	0.840	660	655	87	790

1-Butanol	71-36-3	264	2.404	660	662	56	951
2-Methylbutanal	96-17-3	268	0.840	664	658	58	904
Butyronitrile	109-74-0	276	1.088	672	680	41	906
Thiophene	110-02-1	276	1.124	672	677	84	758
1-Penten-3-one	1629-58-9	296	1.180	692	683	55	885
Acetic anhydride	108-24-7	300	0.904	696	706	43	836
3-Ethoxy-1-propene	557-31-3	308	0.836	702	689	58	862
2-Ethylfuran	3208-16-0	312	0.924	704	711	81	937
Trichloroethylene	79-01-6	312	1.044	704	693	130	868
2,3-Pentanedione	600-14-6	312	1.328	704	700	100	819
3-Pentanol	584-02-1	320	1.644	708	693	59	928
3-Hydroxy-2-butanone	513-86-0	336	0.140	717	705	45	886
Propyl acetate	109-60-4	336	0.964	717	712	61	928
Acetic acid	64-19-7	348	1.112	723	709	45	893
Methyl butanoate	623-42-7	352	0.972	725	720	74	828
3-Methyl-3-buten-1-ol	763-32-6	372	2.960	735	743	68	896
4-Methyl 2-pentanone (Methyl iso-butyl ketone)	108-10-1	384	0.996	742	733	100	852
2-Methyl-1-butanol	137-32-6	384	2.420	742	731	53	909
(2E)-2-Methyl-2-butenal	1115-11-3	392	1.300	746	741	84	888
(2E)-2-Pentenal	1576-87-0	416	1.428	758	751	83	789
2-Methyl-2,4-hexadiene	28823-41-8	428	0.752	765	751	81	801
Toluene	108-88-3	436	1.012	769	766	91	836
1-Pentanol	71-41-0	440	2.428	771	775	42	927
2-Ethoxyethanol	110-80-5	444	2.188	773	744	59	889
2-Methyl-1-propyl acetate (Isobutyl acetate)	110-19-0	452	0.928	777	782	56	930
3-Methyl-2-buten-1-ol	556-82-1	456	3.260	779	767	71	861
(2E)-2-Pentenal	1576-87-0	480	1.652	792	765	84	843
Cyclopentanone	120-92-3	488	1.524	796	791	55	959
(3Z)-3-Hexenal	6789-80-6	504	1.248	803	800	69	897
Ethyl butanoate	105-54-4	508	0.924	805	806	88	864
Hexanal	66-25-1	508	1.044	805	801	56	852
2-Methyldihydro-3(2H)-furanone	3188-00-9	524	1.964	812	812	100	800
Propyl propanoate	106-36-5	528	0.912	814	810	75	929
Butyl acetate	123-86-4	540	0.992	819	819	73	938
2-Cyclopenten-1-one	930-30-3	592	2.460	841	835	82	814
4-Hydroxy-4-methyl-2-pentanone (Diacetone)	123-42-2	604	2.564	847	851	43	940
(2E)-2-Hexenal	6728-26-3	612	1.264	850	847	83	904
(3E)-3-Hexen-1-ol	928-97-2	628	2.336	857	856	67	846
(3Z)-3-Hexen-1-ol	928-96-1	640	2.476	862	866	67	922
Ethylbenzene	100-41-4	644	0.960	864	857	91	948

2-Methylpropyl propanoate	540-42-1	660	0.856	871	866	57	794
(2E)-2-Hexen-1-ol	928-95-0	660	2.592	871	864	57	925
1,2-Dimethylbenzene (o-Xylene)	95-47-6	664	0.960	872	886	91	949
5-Methyl-2(3H)-furanone	591-12-8	664	2.820	872	885	98	799
1-Hexanol	111-27-3	668	2.116	874	867	84	841
2-Methylbutyl acetate	624-41-9	684	1.028	881	873	70	941
4-Penten-1-yl acetate	1576-85-8	696	1.072	886	890	68	872
Styrene (Ethenylbenzene)	100-42-5	712	1.260	893	891	104	945
Cyclohexanone	108-94-1	720	1.412	897	901	98	898
Propyl butanoate	105-66-8	728	0.864	900	895	71	846
2-Heptanone	110-43-0	732	0.988	902	893	58	829
Heptanal	111-71-7	736	0.984	904	906	57	779
2-Butoxyethanol	111-76-2	748	2.012	909	903	57	871
Butyl propanoate	590-01-2	752	0.872	911	910	87	949
(2E,4E)-2,4-Hexadienal (Sorbic aldehyde)	142-83-6	760	1.920	915	914	81	921
Pentyl acetate	628-63-7	764	0.936	917	915	61	953
3-Methyl-2-buten-1-ol acetate (3-Methylbut-2-en-1-yl acetate, Prenyl acetate)	1191-16-8	780	1.100	924	920	67	810
Methoxy-phenyl-oxime	na	780	2.056	924	897	151	779
gamma-Butyrolactone (Dihydro-2(3H)-furanone, 1,2-Butanolide)	96-48-0	792	0.460	930	941	42	920
Propyl 2-methylbutanoate	37064-20-3	832	0.808	948	946	103	853
2-Propenylbenzene (Allyl benzene)	300-57-2	836	1.060	950	953	118	838
3-Methyldihydro-2(3H)-furanone (2-Methylbutanolide)	1679-47-6	840	3.276	952	941	41	858
Butyl 2-methylpropanoate	97-87-0	848	0.804	956	952	89	819
1-Ethyl-2-methylbenzene (2-Ethyltoluene)	611-14-3	864	0.928	963	973	105	822
Benzaldehyde (Phenylmethanal)	100-52-7	872	2.284	967	964	106	939
5-Ethyl-2(5H)-furanone (4-Hydroxy-2-hexenoic acid lactone, 2-Hexen-4-olide)	2407-43-4	872	3.092	967	984	83	802
5-Ethyl-2(3H)-furanone (2-Ethylbutenolide, 3-Hexenoic acid, 4-hydroxy-, gamma-lactone)	2313-01-1	880	2.196	970	954	112	795
1-Heptanol	111-70-6	888	1.708	974	969	70	911
1-Ethyl-4-methylbenzene (4-Ethyltoluene)	622-96-8	892	0.940	976	969	105	907
1-Octen-3-one	4312-99-6	904	1.064	981	975	70	910
1-Octen-3-ol	53907-72-5	908	1.604	983	983	57	840

(1-Methylethenyl)benzene (Isopropenylbenzene)	98-83-9	912	1.132	985	988	118	870
6-Methyl-5-hepten-2-one	110-93-0	916	1.140	987	986	108	822
Methoxymethylbenzene (alpha-Methoxytoluene)	538-86-3	924	1.320	991	984	91	926
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	10230-62-3	924	2.172	991	989	101	853
Benzonitrile (Cyanobenzene)	55-21-0	924	2.708	991	992	103	885
Furfuryl acetate	623-17-6	932	2.060	994	991	81	934
1,2,3-Trimethylbenzene (Hemimellitene)	526-73-8	936	0.976	996	1018	120	835
Butyl butanoate	109-21-7	940	0.844	998	999	71	951
1-Methyl-3-vinylbenzene (m-Methylstyrene)	100-80-1	940	1.164	998	973	118	916
Phenol	108-95-2	940	1.240	998	998	94	842
3-Octanol	589-98-0	944	1.252	1000	999	59	854
Ethyl hexanoate	123-66-0	948	0.856	1002	1003	88	789
1-Ethenyl-2-methylbenzene (2-Methylstyrene)	611-15-4	948	1.152	1002	991	118	903
Benzofuran (Coumarone)	271-89-6	948	1.788	1002	996	118	828
Octanal	124-13-0	956	0.964	1006	1006	84	823
(3Z)-3-Hexenyl acetate	3681-71-8	960	1.012	1008	1008	67	942
2-(2-Ethoxyethoxy)ethanol (Diethylene glycol monoethyl ether)	111-90-0	968	2.580	1012	1006	45	940
Cyclopropylbenzene (Phenylcyclopropane)	873-49-4	972	1.128	1014	1010	118	905
Hexyl acetate	142-92-7	976	0.916	1016	1012	84	894
(2E)-2-Hexenyl acetate	2497-18-9	976	1.036	1016	1019	67	927
1,3-Dichlorobenzene (m-Dichlorobenzene)	541-73-1	980	1.380	1018	1015	146	796
1-Methoxy-4-methylbenzene (4-Methoxytoluene, 4-Methylanisole, p-Methylanisole)	104-93-8	992	1.328	1024	1022	122	914
2,3-Dihydro-1H-indene	496-11-7	1008	1.180	1033	1034	118	794
2-Ethylhexanol	104-76-7	1008	1.472	1033	1028	57	898
Butyl 2-methylbutanoate	15706-73-7	1032	0.800	1045	1047	103	888
Benzenemethanol (Benzyl alcohol, Phenylmethanol)	100-51-6	1032	2.064	1045	1037	108	838
Benzeneacetaldehyde (2-Phenylethanal, Phenylacetaldehyde)	122-78-1	1040	2.280	1049	1045	91	886
Indene	95-13-6	1044	1.392	1051	1045	116	817
(3Z)-3-Octen-1-ol	20125-84-2	1048	1.688	1053	1054	67	828
5-Ethyldihydro-2(3H)-	695-06-7	1060	2.708	1059	1072	85	900

furanone (4-Ethyl-4-butanolide, gamma-Hexalactone)							
1-Methyl-4-propylbenzene (4-Propyltoluene)	1074-55-1	1064	0.888	1061	1056	105	811
Butylbenzene (1-Phenylbutane)	104-51-8	1068	0.896	1063	1068	134	800
(2E)-2-Octenal	2363-89-5	1068	1.152	1063	1062	70	946
1-Octanol	111-87-5	1092	1.520	1076	1074	70	882
(5Z)-5-Octen-1-ol	64275-73-6	1092	1.840	1076	1051	67	818
4-Methylbenzaldehyde (p-Methoxybenzaldehyde, p-Tolualdehyde)	104-87-0	1092	1.896	1076	1076	91	849
1-Ethyl-2,4-dimethylbenzene (4-Ethyl-m-xylene)	874-41-9	1116	0.944	1088	1084	119	785
1-Vinyl-3-ethylbenzene (3-Ethylstyrene)	7525-62-4	1120	1.080	1090	1064	117	944
Pentyl butanoate	540-18-1	1132	0.836	1096	1094	71	856
1-Ethenyl-4-ethylbenzene (4-Ethylstyrene, p-Ethylstyrene)	3454-07-7	1140	1.076	1100	1072	117	936
Nonanal	124-19-6	1156	0.940	1109	1107	57	922
(2E,4E)-2,4-Octadienal	30361-28-5	1168	1.464	1115	1111	81	790
1,3-Diethenylbenzene (m-Vinylstyrene)	108-57-6	1176	1.344	1120	1091	130	923
Benzeneethanol (2-Phenylethanol, Mellol)	60-12-8	1180	0.168	1122	1118	92	936
Butyl 3-hydroxybutanoate	53605-94-0	1204	1.912	1135	1111	87	848
1,7-Dioxaspiro[5.5]undecane	180-84-7	1212	0.924	1139	1108	101	842
Pentyl 2-methylbutanoate	68039-26-9	1216	0.792	1141	1156	103	792
Hexyl 2-methylpropanoate (Hexyl isobutanoate)	2349-07-07	1232	0.796	1150	1150	89	898
5-Propyldihydro-2(3H)-furanone (gamma-Heptalactone)	616-45-5	1244	2.372	1157	1159	85	794
1-Methoxy-4-vinylbenzene (4-Methoxystyrene, 4-Vinylanisole)	637-69-4	1248	1.588	1159	1159	134	887
2-Phenylpropenal (Atropaldehyde)	4432-63-7	1252	2.284	1161	1161	103	787
Benzyl acetate (Phenylmethyl acetate, (Acetoxymethyl)benzene)	140-11-4	1264	1.784	1167	1167	108	950
4-Ethylbenzaldehyde (p-Ethylbenzaldehyde)	4748-78-1	1268	1.684	1170	1164	134	889
1-(4-Methylphenyl)ethanone (1-Methyl-4-acetylbenzene,	122-00-9	1308	1.868	1191	1183	119	819

4'-Methylacetophenone)							
Hexyl butanoate	2639-63-6	1312	0.824	1193	1195	89	916
Naphthalene	91-20-3	1312	1.688	1193	1179	128	833
1-Methoxy-4-(2-propenyl)benzene (4-Allylanisole, Estragole)	140-67-0	1328	1.356	1202	1201	148	945
Decanal	112-31-2	1340	0.920	1210	1208	57	892
Benzothiazole	95-16-9	1384	2.624	1236	1220	135	751
Hexyl 2-methylbutanoate	10032-15-2	1392	0.784	1240	1239	103	932
Phenylethyl acetate	103-45-7	1424	1.632	1260	1257	104	904
4-Methoxybenzaldehyde (4-Anisaldehyde)	123-11-5	1428	2.924	1262	1252	135	936
Hexylbenzene	1077-16-3	1436	0.868	1267	1251	92	820
1,3-Octanediol	23433-05-8	1440	0.168	1269	1275	75	850
Nonanoic acid	112-05-0	1444	1.132	1271	1273	87	812
(2E)-2-Methyl-3-phenyl-2-propenal (alpha-Methylcinnamaldehyde)	101-39-3	1456	1.876	1279	1309	146	833
1-(4-Ethylphenyl)ethanone (4'-Ethylacetophenone, p-Acetylethylbenzene)	937-30-4	1476	1.704	1290	1274	133	874
1-Methoxy-4-propenylbenzene (Anethole, Isoestragole)	104-46-1	1480	1.512	1293	1288	148	949
4-Methoxybenzenemethanol (Anisyl alcohol)	105-13-5	1480	1.832	1293	1295	138	755
(2E)-2-Decen-1-ol	22104-80-9	1496	2.868	1303	1283	57	780
Tridecane	629-50-5	1500	0.648	1305	1300	57	926
1-Methylnaphthalene	90-12-0	1504	1.560	1308	1296	141	812
Undecanal	112-44-7	1512	0.904	1313	1296	82	847
(3E)-4-Phenyl-3-buten-2-one (trans-Benzalacetone)	122-57-6	1512	2.160	1313	1346	131	804
(2E,4E)-2,4-Decadienal	2363-88-4	1528	1.336	1323	1322	81	762
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	74367-34-3	1616	1.304	1378	1376	71	778
Butyl benzoate	136-60-7	1620	1.296	1380	1354	105	753
1,1'-Biphenyl (Lemonene, Phenylbenzene)	92-52-4	1636	1.672	1390	1380	154	926
1,2-Dimethoxy-4-(2-propenyl)benzene (1,2-Dimethoxy-4-allyl benzene, Methyl eugenol)	93-15-2	1656	1.660	1403	1403	178	922
Tetradecane	629-59-4	1660	0.660	1405	1400	57	933
2-Methyl-1,1'-biphenyl (2-Phenyltoluene)	643-58-3	1660	1.380	1405	1395	167	902
Dodecanal	112-54-9	1672	0.920	1413	1425	57	931

1,4-Dimethylnapthalene	571-58-4	1696	1.552	1429	1423	141	792
1,3-Dimethylnaphthalene	575-41-7	1704	1.528	1434	1425	141	860
1-(4-Methoxyphenyl)-1-propanone (4'-Methoxypropiophenone, p-Methoxypropiophenone)	121-97-1	1736	2.268	1455	1484	135	756
1,4-Diacetylbenzene (p-Acetylacetophenone)	1009-61-6	1736	3.404	1455	1451	147	880
Chlorododecane	112-52-7	1768	0.800	1476	1446	91	888
1-Dodecanol	112-53-8	1768	1.220	1476	1473	83	924
4-Methyl-1,1'-biphenyl	644-08-6	1792	1.580	1492	1493	168	923
1,2-Dimethoxy-4-[(1E)-1-propenyl]benzene (trans-4-Propenylveratrole, trans-Methylisoeugenol)	6379-72-2	1800	1.836	1497	1495	178	880
Pentadecane	629-62-9	1808	0.664	1503	1500	57	911
4-Methyl-1,1'-biphenyl	644-08-6	1808	1.580	1503	1493	168	918
Tridecanal	10486-19-8	1824	0.916	1514	1519	82	na
(2-Phenylethyl)benzene (Dihydrostilbene)	103-29-7	1844	1.476	1529	1520	91	939
Dibenzofuran (2,2'-Biphenylene oxide)	132-64-9	1844	2.052	1529	1526	168	755
Dodecanoic acid	143-07-7	1888	3.472	1560	1558	73	881
Hexadecane	544-76-3	1948	0.672	1603	1600	57	897
Tetradecanal	124-25-4	1964	0.912	1615	1610	82	913
(1-Butylheptyl)benzene	4537-15-9	1992	0.812	1636	1626	91	763
Benzophenone (Diphenylmethanone)	119-61-9	1996	2.372	1639	1635	105	891
Tributyl phosphate	126-73-8	2004	1.156	1645	1647	99	773
1-Tetradecanol	112-72-1	2052	1.148	1682	1676	83	876
Heptadecane	629-78-7	2084	0.672	1706	1700	57	897
Pentadecanal	2765-11-9	2100	0.908	1719	1713	82	752
(1-Pentylheptyl)benzene (6-Phenyldodecane)	2719-62-2	2116	0.812	1731	1719	91	834
Nonylphenol	25154-52-3	2132	3.160	1744	1733	107	805
(1-Propylnonyl)benzene (4-Phenyldodecane)	2719-64-4	2140	0.820	1750	1735	91	782
Tetradecanoic acid	544-63-8	2152	2.856	1759	1760	60	894
Hexadecanal	629-80-1	2228	0.912	1820	1811	82	938
Pentadecanoic acid	1002-84-2	2276	2.656	1860	1851	60	836
1-Hexadecanol	36653-82-4	2308	1.108	1887	1882	83	822
7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	82304-66-3	2336	1.952	1904	1929	205	780
Hexadecanoic acid	57-10-3	2396	2.452	1926	1925	60	915

1-Heptadecanol	1454-85-9	2536	1.088	1976	1986	111	864
Heptadecanoic acid	506-12-7	2616	2.268	2007	1977	60	na

$^{1}t_R$ and $^{2}t_R$ – first and second dimension retention times, respectively
RI_{exp} – experimental linear temperature-programmed retention index
RI_{lit} – literature linear temperature-programmed retention index
na – not available
SIM – mass spectral similarity

Table S-5. Comparison between *ex vivo* and *in vivo* SPME

consideration	*ex vivo* SPME	*in vivo* DI-SPME
sample preparation	requires metabolism quenching and more extensive sample preparation	metabolism quenching requirements eliminated
extraction method	requires extraction phase chemistries compatible with complex matrix and allowing clean-up of the coating (DI-SPME);	requires extraction phase chemistries compatible with complex matrix and allowing clean-up of the coating;
	matrix effects and production of volatile end-products of Maillard reaction in GC inlet (DI-SPME)	matrix effects and enhanced production of volatile end-products of Maillard reaction in GC inlet
reproducibility in extraction	unstable metabolites require fresh sample preparation;	improved reproducibility for unstable metabolites;
	lower reproducibility due to adsorptive losses of hydrophobic metabolites	improved reproducibility for hydrophobic metabolites;
		extraction efficiency related to sampling position
metabolome coverage	biased against non volatile and polar metabolites (HS-SPME);	rich metabolite coverage;
	capture of polar and hydrophobic classes of metabolites (DI-SPME)	capture of polar and hydrophobic classes of metabolites;
instrument	less enhanced introduction of non volatile matrix results in cleaner chromatograms, improved metabolite detectability and faster data processing;	more enhanced introduction of non volatile matrix results in complex chromatographic profiles; multi-dimensional instruments required to improve metabolite deconvolution from matrix and data processing;
	introduction of water and contamination due to matrix requires frequent replacement of second dimension column (DI-SPME);	introduction of water and contamination due to matrix requires frequent replacement of second dimension column;
	higher thickness of stationary phase required in second dimension (DI-SPME)	higher thickness of stationary phase required in second dimension

References

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