Combination of SPME as non-invasive sample preparation technique and GCxGC-TOFMS for high resolution profiling of metabolites in apples: method development considerations and potential of new *invivo* SPME formats

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Introduction

Solid phase microextraction: SPME

developed by Pawliszyn et al. in 1989

SPME features

↓ disadvantages of traditional sample preparation;

combination of sampling, extraction, concentration & sample introduction into 1 solvent free step;

HS- & DI-SPME extraction modes of gaseous, liquid and solid samples;

↓ sample amount, ↓ sample preparation times & easy automation

MINIATURIZED FORMAT & NON-EXHAUSTIVE EXTRACTION

↓ disturbance to investigated biological system;
+
invivo sampling:
metabolism quenching step eliminated

more representative sample extracts;
more representative metabolism snapshot;
↓ sample preparation, extraction & storage artifacts;
detection of rapid and short metabolism changes

Project Objectives

SPME coating selection for GC-metabolomics

metabolite profiling of apples using SPME-GCxGC-TOFMS

comparison between *invivo* & *exvivo* sampling formats

metabolite alignment, statistical analysis & identification of *biomarkers*

1 2 3

harvest & postharvest apple quality

Experimental

Pegasus 4D GCxGC-TOFMS system (LECO, St. Joseph, MI, USA):

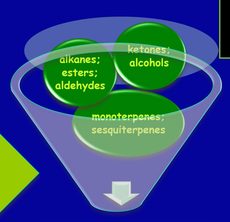
Agilent 6890N GC
high-speed TOF
dual-stage quad-jet cryogenic modulator from Zoex &
MPS 2 autosampler from Gerstel

SPME coating selection

comprehensive evaluation:

extraction selectivity, extraction sensitivity & desorption efficiency of SPME coatings across a volatility & polarity range

MW range
88.15-312.54 g/mol;
boiling point range
115.64-360.59 °C;
log K_{ow} range
1.26-8.72



3 mL water sample spiked with 52 compounds belonging to homologous series

GCxGC-TOFMS conditions: metabolomics samples

RXI-5 SIL MS x Supelcowax/BP20/Stabilwax columns,
1.5 mL/min flow rate,
40 °C (5 min), 3 °C/min to 240 °C (10 min),
10 °C secondary oven offset,
30 °C modulator temperature offset,
5 sec modulation, 1 sec hot pulse time,
m/z 33-550 acquisition range at 200 spectra/sec,
1700 V detector voltage

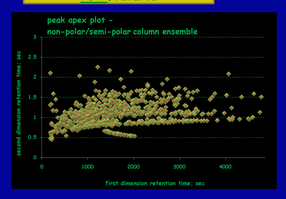
SPME sampling protocols

exvivo	vs	*invivo*
i) *metabolism quenching* liquid nitrogen; saturated NaCl solution		i) *1 hr DI-SPME sampling*
ii) *homogenization*		ii) *wash step in water after sampling & before desorption*
iii) *1 hr HS-SPME & DI-SPME sampling*		iii) *desorption & GCxGC-TOFMS analysis*
iv) *desorption & GCxGC-TOFMS analysis*		

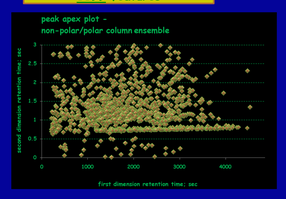
Results & Discussion

GCxGC column combinations

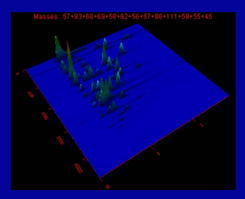
RXI-5Sil MS/DB-17; 781 features



RXI-5Sil MS/ SUPELCOWAX; 1199 features

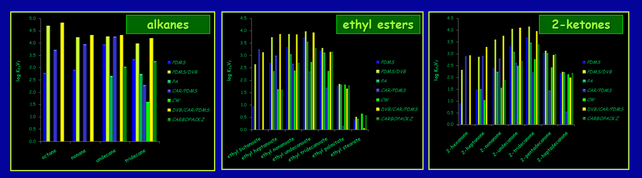


SPME coating selection: extraction sensitivity



1.5 mL/min
flow rate;
35 °C (5 min) initial & 245 °C (3 min) final
temp for 1st dimension;
3 °C/min rate
15 °C
2nd oven temp offset;
35 °C
modulator temp offset;
3 sec
modulation period;
0.6 sec
hot pulse time

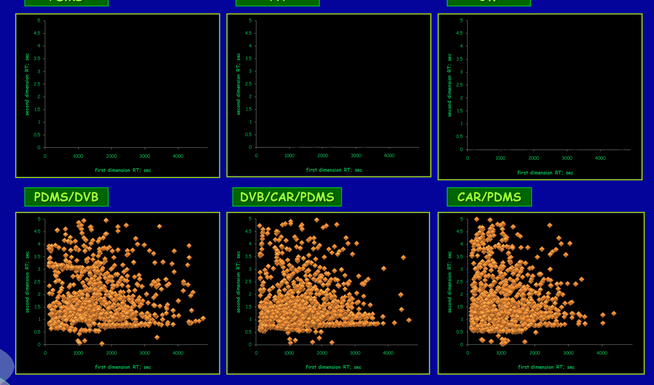
calculation of fibre coating/sample matrix distribution constants (K_{fs}) & fibre constants (K_{fs}, V_f)



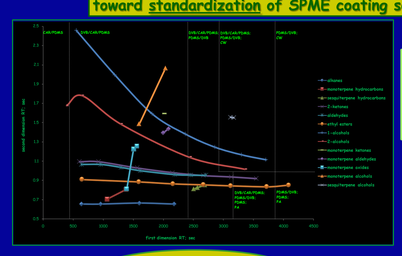
spiked water samples

1 hour HS-SPME extraction;
30 °C sample temperature;
10 min desorption @ 5 °C < max temp

SPME coating selection: extraction selectivity



toward standardization of SPME coating selection



MW & log K_{ow} thresholds:

best sensitivity, selectivity & desorption efficiency

liquid sorbents

i) non-polar compounds (alkanes and monoterpene hydrocarbons), slightly polar volatile compounds (ethyl esters, MW < 187 g/mol): **PDMS**
ii) more polar components (aliphatic aldehydes and ketones), sesquiterpene hydrocarbons: performance characteristics of **PDMS** and **PA** are not statistically different
iii) polar components having log K_{ow} < 3.30 (1-alcohols and 2-alcohols): **CW** and **PA**

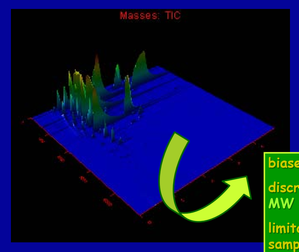
solid sorbents

i) MW < 80-90 g/mol: **CAR/PDMS**
ii) MW 90-250 g/mol: **DVB/CAR/PDMS**
iii) MW 185-315 g/mol: **PDMS/DVB**

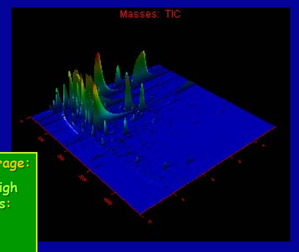
exvivo & invivo sampling

Δ in SPME sampling protocols: metabolite coverage

exvivo HS-SPME # of features (S/N 100): 2413

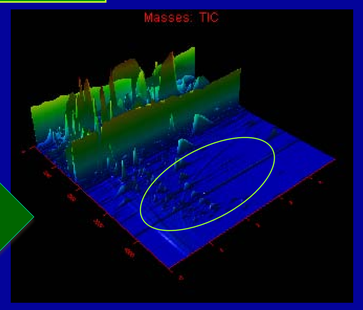


exvivo DI-SPME # of features (S/N 100): 4297



biased metabolite coverage:
discrimination toward high MW & polar metabolites:
limitation of HS-SPME sampling

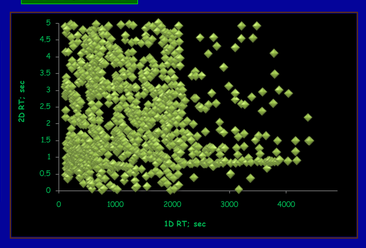
invivo DI-SPME # of features (S/N 100): 8344



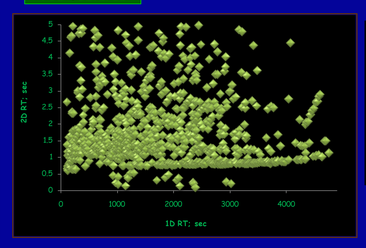
↑ metabolite coverage for high MW and polar metabolites

post-processing of peak tables for DI-SPME: S/N 200

invivo 1048 features

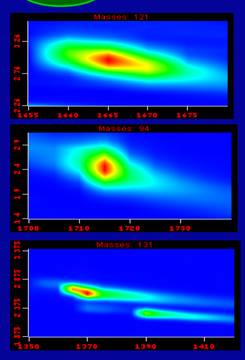


exvivo 906 features

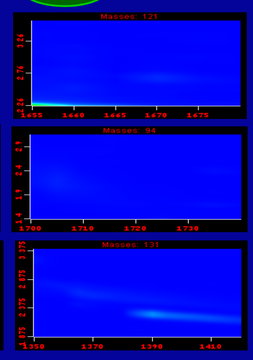


Δ in SPME sampling protocols: differential metabolites *invivo*

invivo



exvivo



2,4,6-trimethylphenol

2-phenoxyethanol

2-methylbenzofuran

enzyme activation during *exvivo* sample preparation step: non-specific degradation of metabolites ?

differential metabolites table

analyte name	CAS #	RI_{exp}	RI_{lit}	similarity	structure
2,4,6-Trimethylphenol	527-60-6	1205	1204	824	
2-Phenoxyethanol	122-99-6	1223	1226	898	
gamma-Butyrolactone	96-48-0	944	941	965	
2-Methylbenzofuran	4265-25-2	1105	1109	879	
2,2'-Bifuran	5905-00-0	1037	1047	872	
Butyl stearate	123-95-5	2385	2374	862	
2-(2-Ethoxyethoxy)ethanol	111-90-0	1019	1006	953	
Z-11-Hexadecenoic acid	2416-20-8	1938	1953	863	
(2-Phenoxyethoxy)benzene	104-66-5	1793	1811	892	

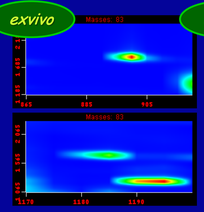
Δ in SPME sampling protocols: differential metabolites *exvivo*

autoxidation/lipid peroxidation of unsaturated fatty acids and unsaturated acyl lipids

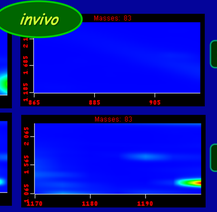
precursor: linoleic acid

3 differential metabolites

exvivo



invivo



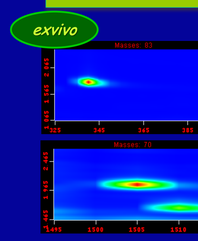
(2E)-2-Heptenal

(2Z)-2-Octenal

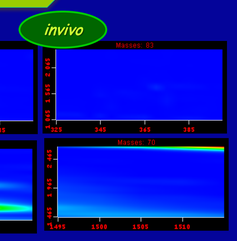
precursor: linolenic acid

5 differential metabolites

exvivo



invivo

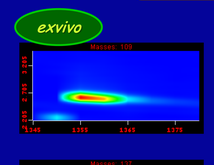


(2E)-2-Pentenal

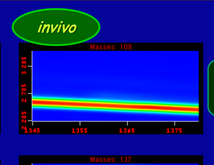
(2E,6Z)-2,6-Nonadienal

oxidative degradation of carotenoids

exvivo



invivo



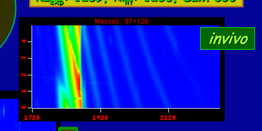
6-Methyl-3,5-heptadiene-2-one
PRECURSOR: Dehydrolycopene

β-cyclocitral
PRECURSOR: β-Carotene

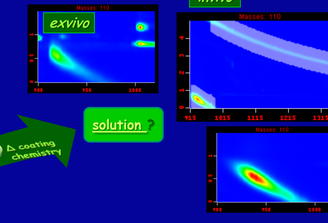
invivo sampling: CHALLENGES

Maillard rxn artifacts generation during desorption step

2-hydroxymethyl-5-furfural, RI_{exp}: 1259, RI_{lit}: 1256, SIM 856



5-methylfurfural, RI_{exp}: 967, RI_{lit}: 960, SIM 886



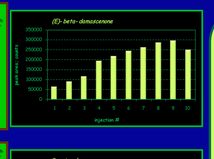
solution ?

Δ coating chemistry
wash step optimization
acquisition rate ↑

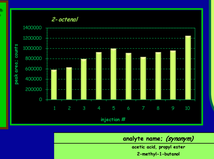
Δ in SPME sampling protocols: precision

exvivo HS-SPME
no fibre fouling

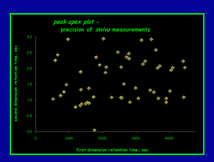
precision of HS-SPME
same aliquot;
↓ aging effects:
median RSD: 6.4 %
minimum: 0.5 %
maximum: 23.1 %



precision of HS-SPME & sample prep
different aliquots;
aging effects:
median RSD: 13.6 %
minimum: 2.5 %
maximum: 88.6 %

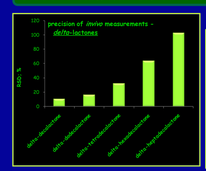


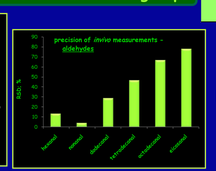
invivo DI-SPME
intra-fruit repeatability;
n = 3 fibers, 53 compounds

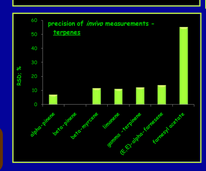


precision of invivo DI-SPME
median RSD: 16.3 %
minimum: 0.3 %
maximum: 102.3 %

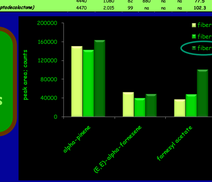
close examination of selected functional groups

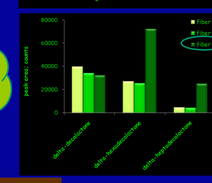






↑ RSD
↑ BPt in homologous series





inter-fibre performance

EXVIVO:
excellent inter-fibre repeatability across MW & volatility range

Δ in metabolite distribution ???

analyte name: (synonym)	$^1P_{fs}$; sec	$^2P_{fs}$; sec	*m/z*	SIM RI_{exp}	RI_{lit}	RSD; %
acetic acid, propyl ester	525	1.020	61	726	ns	13.7
2-methyl-1-butanol	588	2.240	70	945	741	25.9
1-pentanol	646	2.420	70	948	770	28.4
hexanal	759	1.135	56	927	803	801	13.2
2-vinyl-5-methylfuran	852	1.250	81	931	831	ns	24.7
2-hexenal	930	1.475	69	961	855	850	20.8
trans-2-hexenol	972	2.505	57	957	867	ns	19.3
2,6,6-trimethylbicyclo[3.1.1]hept-2-ene, (alpha-pinene)	1094	0.760	93	891	934	933	7.2
6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane, (beta-pinene)	1144	0.825	93	928	979	978	0.3
1-octen-3-ol	1247	1.885	57	937	980	978	11.7
6-methyl-5-hepten-2-one	1262	1.320	108	895	986	986	9.3
2-methyl-6-methylene-2,7-octadiene, (beta-myrcene)	1280	0.865	93	877	990	991	11.7
1-methyl-4-(1-methylethenyl)cyclohexene, (limonene)	1309	0.870	68	894	1030	1031	11.0
butyl 2-methylbutanoate	1545	0.905	103	911	1042	ns	23.6
2-octenal	1596	1.365	55	950	1058	1059	3.7
1-isopropyl-4-methyl-1,4-cyclohexadiene, (gamma-terpinene)	1652	0.890	93	870	1059	1058	12.2
nonanal	1741	1.090	57	958	104	1107	4.1
cis,cis-4,6-octadienol	1767	0.035	67	802	1112	ns	14.4
butyl-3-hydroxybutanoate	1838	2.345	87	854	1229	ns	16.9
4-ethylbenzaldehyde	1920	2.330	134	894	1164	1161	9.0
(2E)-3-phenyl-2-propenal, (trans-cinnamaldehyde)	2024	2.930	131	895	1202	ns	14.0
4-isopropylbenzaldehyde, (cuminaldehyde)	2091	1.855	148	844	1223	ns	6.6
1-methoxy-4-[(E)-1-propenyl]benzene, (trans-anethole)	2124	2.030	78	835	1254	ns	15.0
2-undecanone	2283	1.070	58	844	1291	1294	13.8
5-pentylfuran-2(3H)-furone, (gamma-nonalactone)	2469	2.505	85	894	1361	1362	13.9
2-dodecanone	2596	1.065	58	787	1394	1393	23.4
1,2-dimethoxy-4-(2-propenyl)benzene, (methyl eugenol)	2562	2.030	178	932	1397	1403	21.3
dodecanal	2589	1.060	41	957	1407	1410	28.9
1,3-diacetylbenzene	2646	1.695	147	958	1430	ns	4.1
5-hexyldihydro-2(3H)-furanone, (gamma-decalactone)	2730	2.355	85	751	1465	1469	12.5
6-pentyltetrahydro-2H-pyran-2-one, (delta-decalactone)	2793	2.460	99	854	1494	1494	10.7
1-(4-methoxyphenyl)-2-butanone, (hasterry ketone methyl ether)	2799	2.295	178	883	1493	ns	10.6
1,3,6,10-dodecatetraene, 3,7,11-trimethyl-, ((3E,6E)-, (farnesene (E,E)-, alpha-))	2826	0.975	93	854	1504	1504	13.0
1-tridecanol	2991	1.370	55	873	1574	1580	14.4
tetradecanal	3078	1.040	57	947	1612	1614	46.3
phenyl benzoate	3280	2.890	105	917	1659	ns	25.4
dihydro-5-octyl-2(3H)-furanone, (gamma-dodecalactone)	3219	2.125	85	826	1677	1681	10.7
6-heptyltetrahydro-2H-pyran-2-one, (delta-dodecalactone)	3279	2.220	99	778	1704	1708	16.3
1-pentadecanol	3435	1.305	83	779	1764	1767	14.7
valeric acid, 2-pentadecyl ester	3462	2.935	85	699	1791	ns	34.6
2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, acetate, (farnesyl acetate)	3546	1.245	69	744	1833	1832	55.2
4-(1-methyl-1-phenylethyl)phenol, (4-cumylphenol)	3597	2.575	197	824	1858	ns	6.6
5-decyldihydro-2(3H)-furanone, (gamma-tetradecalactone)	3657	2.000	85	ns	1888	ns	22.6
2-heptadecanone	3681	1.040	58	811	1900	1895	43.0
6-nonyltetrahydro-2H-pyran-2-one, (delta-tetradecalactone)	3727	2.070	99	746	1939	1937	31.2
octadecanal	3909	1.040	96	867	2004	2024	66.4
1-methylethyl hexadecanoate, (isopropyl palmitate)	3932	0.595	60	896	2016	ns	32.8
1-octadecanol	4023	1.275	83	875	2060	ns	60.7
5-undecyldihydro-2(3H)-furanone, (gamma-pentadecalactone)	4056	1.935	85	ns	2073	ns	72.1
6-undecyltetrahydro-2H-pyran-2-one, (delta-hexadecalactone)	4110	2.010	99	ns	2095	ns	63.7
2-ethylhexyl 4-methoxycinnamate	4402	2.220	178	936	ns	ns	48.0
eicosanal	4440	1.080	82	880	ns	ns	77.5
6-docosyltetrahydro-2H-pyran-2-one, (delta-heptadecalactone)	4470	2.035	99	ns	ns	ns	102.3

Conclusions

potential of novel *invivo* SPME sampling formats and GCxGC-TOFMS for unbiased and representative characterization of metabolome
↑ metabolite coverage (1000s of metabolites at required sensitivities);
median intra-fruit RSD - 16.3%;
detection of differential metabolites

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