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Terpenes – properties and determination

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International Conference on Food Safety and Regulatory Measures
August 17-19, 2015 Birmingham, UK

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SUPERFRUITS

"**superfruit**"

- recently introduced to the nomenclature
- comprises **13** natural products (e.g. fruits, vegetables, corns and tea)
- many health benefits
- easily enhance well-being

requirements:

- condensed content of **nutrients**,
- high content of **antioxidants**,
- **health-related properties** proven in research,
- **effects** on the properties of the **cell** and **molecular structures** within the **body**,
- **exotic origin**, interesting taste, smell and appearance

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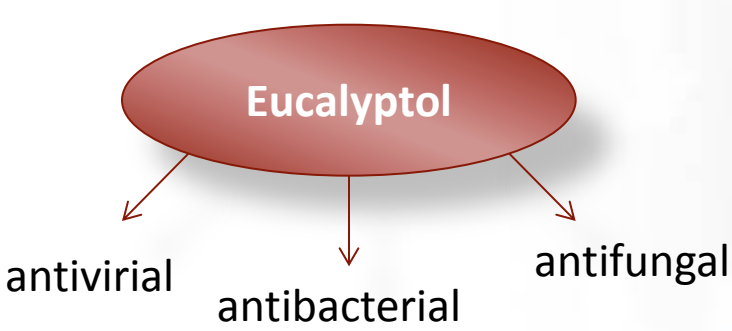
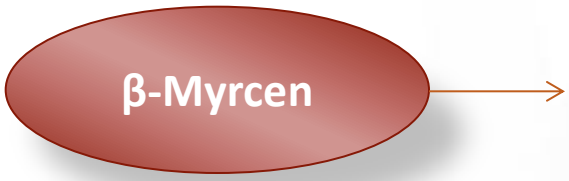
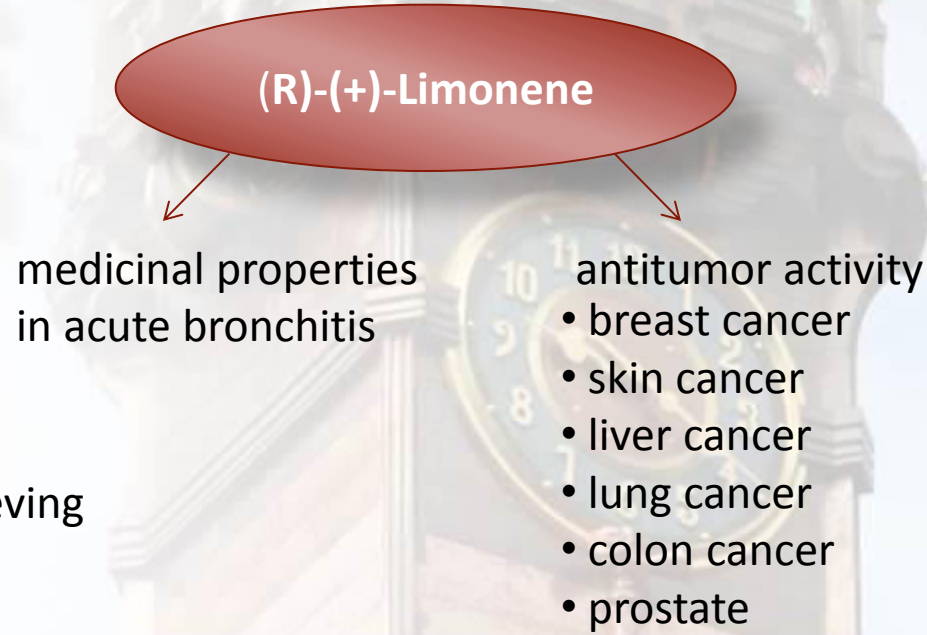
Terpenes

- main group of secondary metabolites - 30 000 compounds
- over 400 monoterpenes known
- characteristic flavour and different biological activity







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Physalis peruviana

Known as:
Cape gooseberry (South Africa),
Inca berry,
Aztec berry,
Golden berry,
Giant ground cherry,
Peruvian groundcherry,
Peruvian cherry,
Pok pok (Madagascar),
Poha (Hawaii),
Ras bhari (India),
Aguaymanto (Peru),
Uvilla (Ecuador),
Uchuva (Colombia),
Harankash (Egypt),
Miechunka Peruwiańska (Poland).

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Cape Gooseberry (*Physalis peruviana*)

- gold juicy beads with size of about 2 cm
- taste of the fruit described as "strawberry / kiwi /gooseberry-like"
- fruit are protected by the surrounding leaves

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Cape Gooseberry (*Physalis peruviana*)

- cultivated in South America (Peru, Colombia Equador), California, South Africa, India, New Zealand, Australia and Egypt

- in Poland:
  - specialized stores with national origin organic food
  - supermarkets as fruits from South America

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Malus Baccata

- a species of apple
- known as:

  Crabapple,

  Siberian crabapple,

  Siberian crab,

  Manchurian crab apple,

  Chinese crab apple,

  Jabłoń jagodowa (Poland)

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Crabapple (*Malus Baccata*)

- native to most of Asia, also grown elsewhere as an ornamental tree and for rootstock
- used for bonsai
- shurb or tree up to 5 m high
- red to yellow fruit with size of about 1 cm
- taste is quite acrid and sour

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Elaeagnus Multiflora

- known as:

  Cherry Silverberry

  Goumi,

  Gumi,

  Natsugumi,

  Oliwnik wielokwiatowy (Poland)

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Cherry Silverberry (*Elaeagnus Multiflora*)

- native to China, Korea and Japan
- semi-evergreen shrub or small tree growing to 2-8 m
- round to oval drupe fruits 1 cm long, silvery-scaled orange, ripening red dotted with silver or brown, pendulous on a 2-3 cm peduncle
- taste of the fruit is characterized as sour and slightly astringent

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Crataegus Coccinea

- common name: ***Scarlet Hawthorn***

  Głóg szkarłatny **(Poland)**

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Scarlet Hawthorn (*Crataegus Coccinea*)

- native to North America
- shurb or tree up to 7 m high
- blood-red fruit with characteristics crater-like cavity and size of about 2 cm
- taste of the fruit is characterized as sweet and sour

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Determination of terpenes and volatile compounds in fruits

International Conference on Food Safety and Regulatory Measures
August 17-19, 2015 Birmingham, UK

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Method

Defrose and mix fruit samples

↓

8 g of sample and 2 g of NaCl to a vial of 20 ml

↓

Thermostating with agitation

↓

Exposure of the SPME fiber in the sample headspace

↓

Thermal desorption of analytes in the two-dimensional gas chromatography injector

↓

Final analysis

- GCxGC with cryogenic modulator (SLB-5ms and IL-60, modulation 6s, *n*-alcanes)
- GCxGC with flow modulator (SLB-5ms and IL-60, modulation 6s, *n*-alcanes)
- GC-MS (SLB-5ms column, *n*-alcanes)
- GC-MS (Supelcowax-10, n-alcanes)
- GC-MS (Supelcowax-10, FAMEs)
- GC-MS (Supelcowax-10, FAEEs)

Data were collected by the GCMS Solution software (Shimadzu)

Two dimensional data were elaborated by using the ChromSquare v.2.1 software (Shimadzu)

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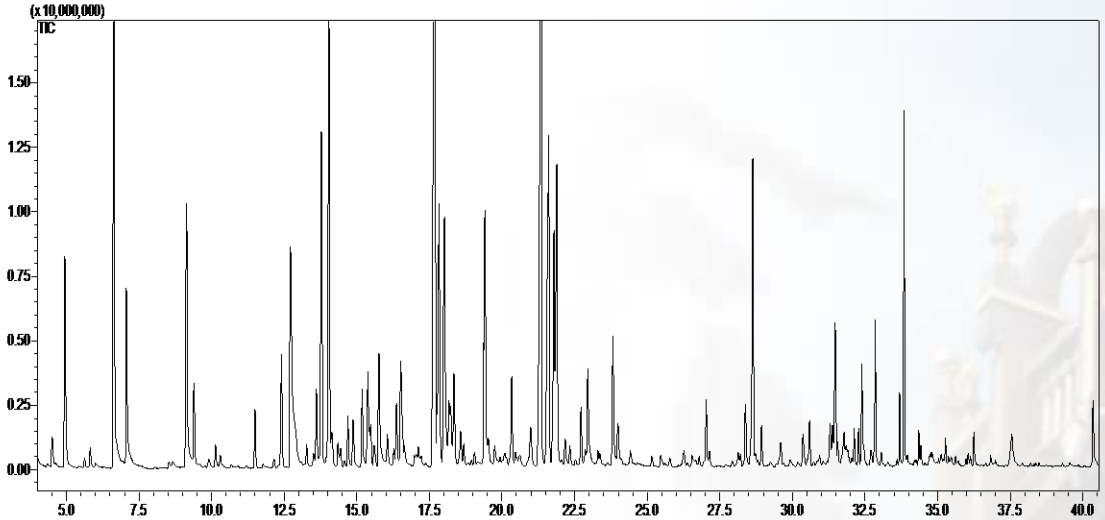
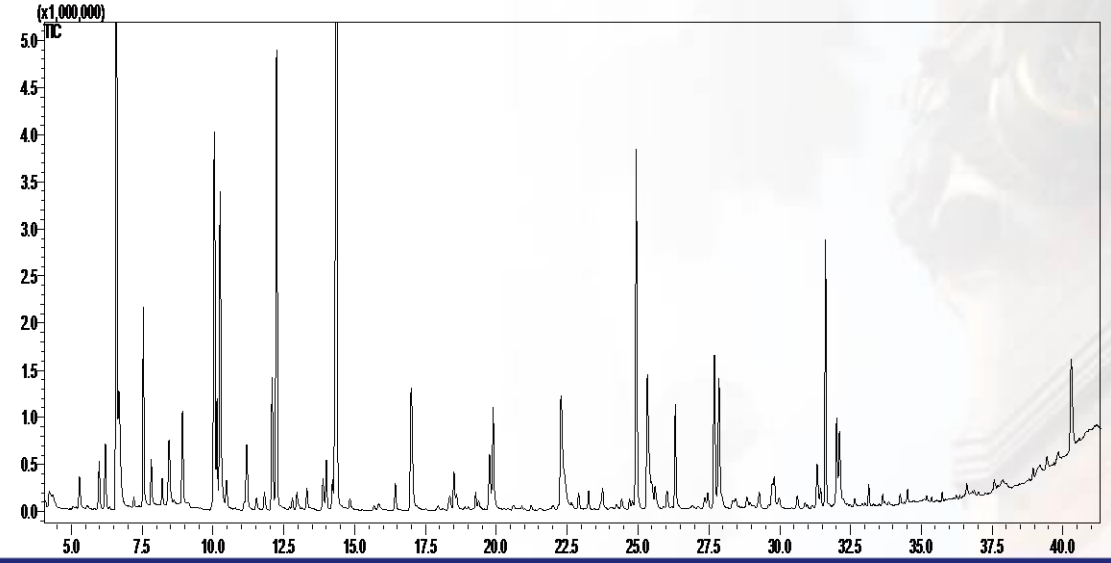
GC-MS



GC-MS
(SLB-5ms column)
chromatogram of HS-SPME
of Cape gooseberry.



GC-MS
(Supelcowax-10 column)
chromatogram of HS-SPME
of Cape gooseberry.

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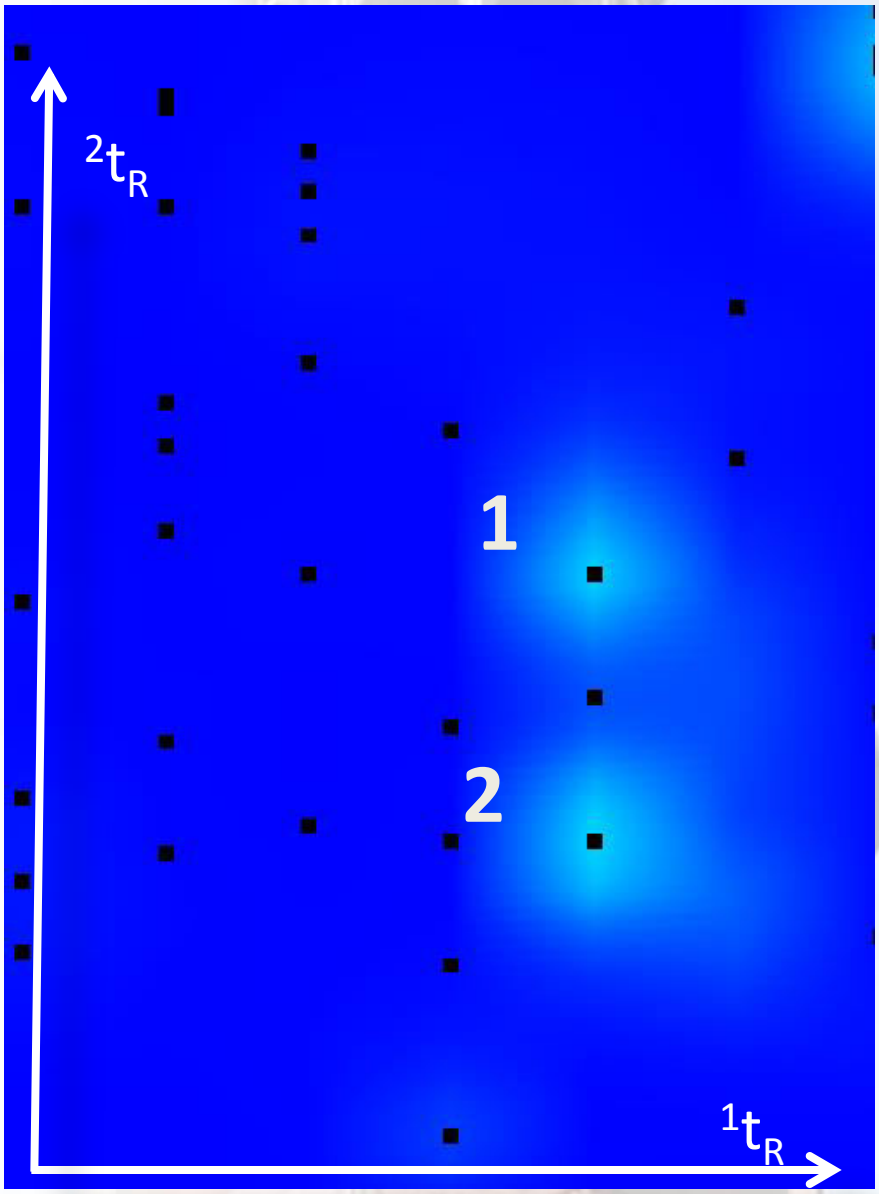
Why GCxGC??



1 - eucalyptol
2 - limonene

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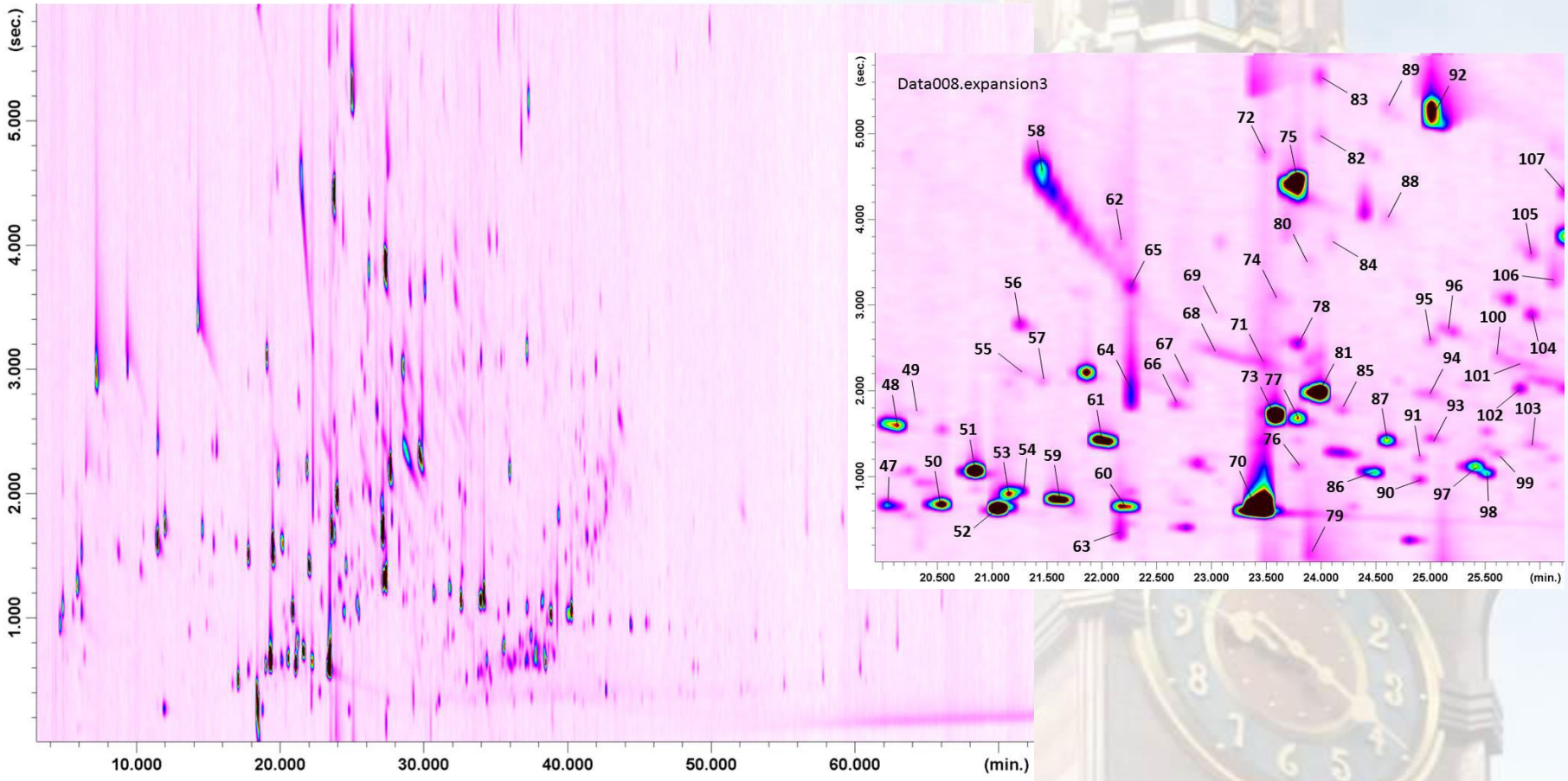
HS-SPME/GC×GC-qMS of Cape gooseberry



2D chromatogram obtained by GC × GC-qMS with cryogenic modulator analyzing of the sample cape gooseberry fruit.

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Optimization of GCxGC and extraction parameters

GCxGC-TOFMS parameters	Optimal value
Modulation time	3s, 4s, 5s, **6s**
Sampling frequency	75Hz, 100Hz, **125Hz**, 200Hz
Final temperature programm	40°C (3min) 5°C/min 150°C (0min) 10°C/min 250°C(2min) total time of analysis 37min

Extraction parameters	Optimal value
Stationary phase of the extraction fiber	PDMS/DVB (65µm) CAR/PDMS (85µm) PDMS (100µm) **DVB/CAR/PDMS (50/30µm)**
Mass of the sample	4g, 6g, **8g**
Desorption time	2min, **3min**, 4min
Extraction temperature	30°C, 40°C, **50°C**
Extraction time	15min, 30min, 45min **(26,5min)**
Incubation time	10min, 20 min, 30min **(28,89min)**
Addition of NaCl	without, 1g, **2g**, 3g

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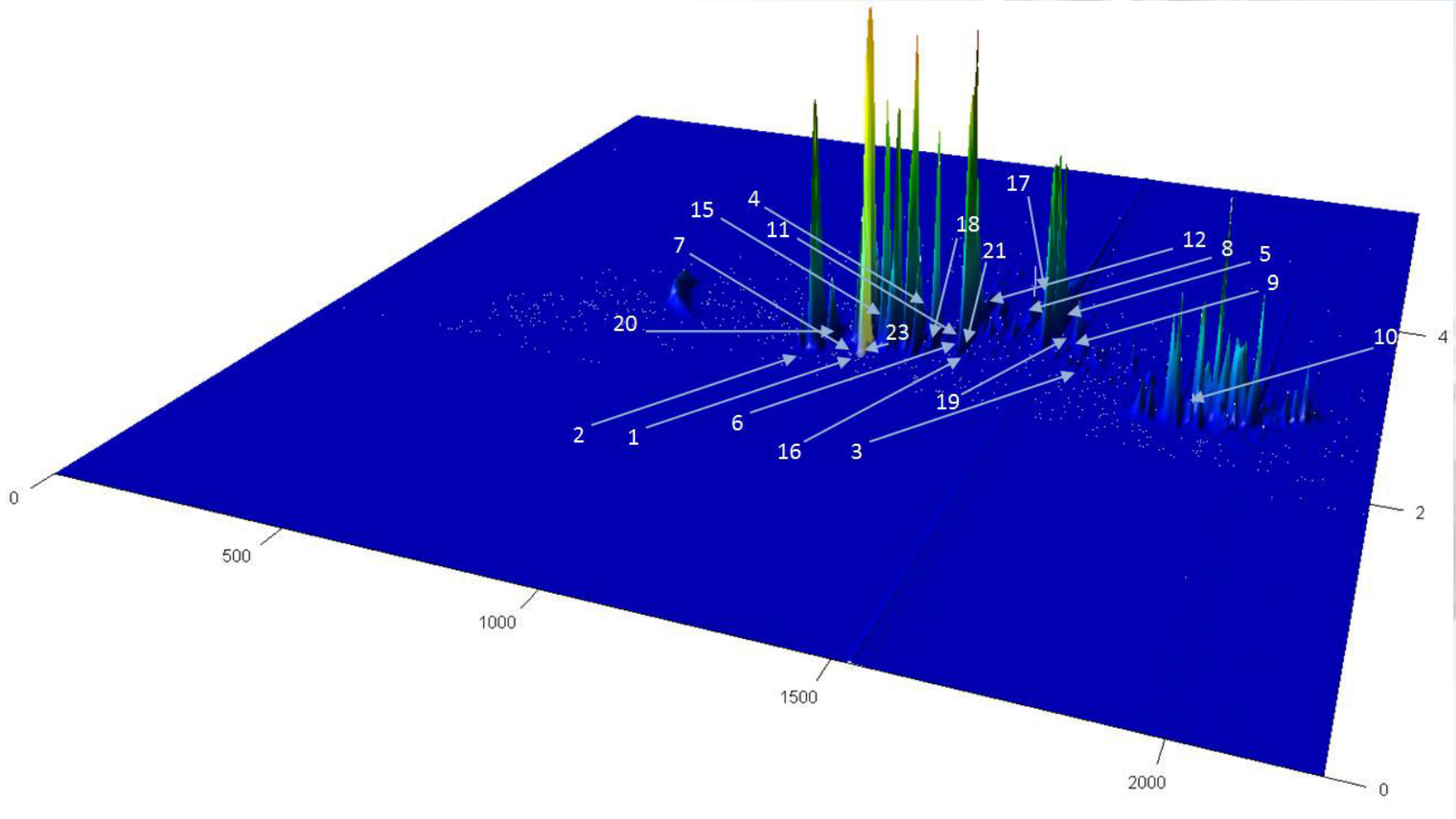
HS-SPME/GC×GC of Cape gooseberry



3D chromatogram obtained by GC × GC-TOFMS analyzing of the cape gooseberry fruit sample

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cape gooseberry
63 terpenes

Formula	Name	$LRI_{lit.}$	$LRI_{calc.}$	^1Dt_R[s]	^2Dt_R[s]
Monoterpene ketones					
$C_{10}H_{16}O$	β-Thujone	1089	1069	1338	2,7
$C_{13}H_{20}O$	α-Ionone	1428	1411	1860	2,7
$C_{10}H_{16}O$	trans-3-Pinanone, (E)-Pinocamphone	1143	1148	1482	3,4
$C_{10}H_{16}O$	**(-)-Camphor**[8]	1127	1131	1452	3,6
$C_{10}H_{14}O$	(-)-Carvone	1212	1228	1614	3,2
$C_{10}H_{16}O$	Pulegone	1211	1222	1506	3,2
$C_{10}H_{14}O$	Pinocarvone	1150	1148	1482	3,6
$C_{10}H_{16}O$	**(-)-Fenchone**[11]	1080	1075	1350	3,3
Monoterpene oxides					
$C_{10}H_{16}O_2$	(1R,4R)-p-Mentha-2,8-diene, 1-hydroperoxide	1324	1337	1764	2,9
$C_{10}H_{12}O_2$	Linalool oxide	1078	1078	1356	3,0
$C_{10}H_{18}O$	trans-Rose oxide	1115	1117	1428	3,0
Monoterpene aldehydes					
$C_{10}H_{16}O$	Citral[9]	1222	1224	1608	3,0
$C_{10}H_{16}O$	Geranial	1270	1252	1650	3,0
$C_{10}H_{16}O$	α-Campholenal	1115	1110	1416	3,3
$C_{10}H_{16}O$	Carvenone	1277	1244	1638	3,2
Sesquiterpenes					
$C_{15}H_{22}$	β-Vatirenene	1452	1441	1926	2,6
$C_{15}H_{24}$	β-Copaene	1418	1446	1938	2,8
$C_{15}H_{24}$	β-Curcumene	1510	1451	1950	2,5
$C_{15}H_{20}$	α-Calacorene	1517	1465	1980	2,7

Formula	Name	$LRI_{lit.}$	$LRI_{calc.}$	^1Dt_R[s]	^2Dt_R[s]
Monoterpene hydrocarbons					
$C_{10}H_{16}$	**β-Pinene**[1]	981	980	1170	2,7
$C_{10}H_{16}$	**α-Pinene**[2]	937	937	1074	2,7
$C_{10}H_{16}$	**R-(+)-Limonene**[23]	1026	1025	1254	2,9
$C_{10}H_{14}$	1,3,8-p-Menthatriene	1105	1103	1404	3,1
$C_{10}H_{18}$	**β-Myrcene**[7]	985	985	1176	2,8
$C_{10}H_{14}$	**p-Cymene**[12]	1036	1078	1356	3,2
$C_{10}H_{16}$	**α-Phellandrene**[15]	1003	1003	1212	2,8
$C_{10}H_{16}$	**Terpinolene**[16]	1084	1084	1368	3,0
$C_{10}H_{16}$	**γ-Terpinene**[18]	1051	1053	1308	2,9
$C_{10}H_{16}$	**Camphene**[20]	952	954	1110	2,7
$C_{10}H_{16}$	α-Thujene	921	923	1176	2,7
$C_{10}H_{16}$	β-Thujene	968	951	1104	2,8
$C_{10}H_{16}$	β-Phellandrene	1021	1025	1254	3,0
$C_{10}H_{16}$	Cyclohexene, 1,5,5-trimethyl-3-methylen-	992	991	1188	2,8
$C_{10}H_{16}O$	**β-Cyclocitral**[19]	1215	1208	1584	3,2
Monoterpenols					
$C_{10}H_{18}O$	**Eucalyptol**[4]	1030	1028	1260	3,0
$C_{10}H_{20}O$	**(-)-Menhtol**[6]	1150	1169	1518	3,0
$C_{10}H_{18}O$	**4-Terpineol**[17]	1172	1172	1524	3,2
$C_{10}H_{18}O$	**(-)-Linalool**[21]	1087	1087.5	1374	3,0
$C_{10}H_{18}$	**Geraniol**[3]	1237	1240	1632	2,9
$C_{10}H_{18}O$	**α-Terpineol**[5]	1182	1183	1542	3,3

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crabapple
39 terpenes

Formula	Name	$LRI_{lit.}$	$LRI_{calc.}$	^1Dt_R[s]	^2Dt_R[s]
Monoterpene hydrocarbons					
$C_{10}H_{16}$	**α-Pinene***	937	937	1074	2,7
$C_{10}H_{16}$	**R-(+)-Limonene***	1026	1025	1254	2,9
$C_{10}H_{14}$	1,3,8-p-Menthatriene	1105	1103	1404	3,1
$C_{10}H_{18}$	**β-Myrcene***	985	985	1176	2,8
$C_{10}H_{14}$	**p-Cymene***	1036	1078	1356	3,2
$C_{10}H_{16}$	**β-trans-Ocimene***	1045	1041	1284	2,8
$C_{10}H_{16}$	**α-Phellandrene***	1003	1003	1212	2,8
$C_{10}H_{16}$	**Terpinolene***	1084	1084	1368	3,0
$C_{10}H_{16}$	**γ-Terpinene***	1051	1053	1308	2,9
$C_{10}H_{16}$	Cyclohexene, 1,5,5-trimethyl-3-methylen-	992	991	1188	2,8
$C_{10}H_{16}$	(+)-3-Carene, 2-(acetylmethyl)-	1390	1399	1896	2,7
$C_{10}H_{16}O$	**β-Cyclocitral***	1215	1208	1584	3,2
Monoterpenols					
$C_{10}H_{18}O$	**Eucalyptol ***	1030	1028	1260	3,0
$C_{10}H_{20}O$	(1S,2R,5R)-(+)-Isomenthol	1164	1166	1512	3,1
$C_{10}H_{18}O$	4-Terpineol	1172	1172	1524	3,2
$C_{10}H_{18}O$	**(-)-Linalool***	1087	1087.5	1374	3,0
$C_{10}H_{18}$	**Geraniol***	1237	1240	1632	2,9
$C_{10}H_{18}O$	**α-Terpineol***	1182	1183	1542	3,3
$C_{10}H_{12}O_2$	**Eugenol***	1345	1337	1764	3,0
$C_{10}H_{16}O$	Myrtenol	1190	1190	1554	3,2
$C_{10}H_{16}O$	Verbenol	brak	1162	1506	3,2
$C_{10}H_{16}O$	Carveol	1192	1197	1566	3,2
$C_{10}H_{18}O_2$	Lilac alcohol C	968	963	1128	2,7

Formula	Name	$LRI_{lit.}$	$LRI_{calc.}$	^1Dt_R[s]	^2Dt_R[s]
Monoterpene ketones					
$C_{13}H_{20}O$	α-Ionone	1428	1411	1860	2,7
$C_{10}H_{16}O$	trans-3-Pinanone,	1143	1148	1482	3,4
$C_{10}H_{16}O$	**(-)-Camphor***	1127	1131	1452	3,6
$C_{10}H_{14}O$	Pinocarvone	1150	1148	1482	3,6
Monoterpene oxides					
$C_{10}H_{12}O_2$	Linalool oxide	1078	1078	1356	3,0
Monoterpene aldehydes					
$C_{10}H_{16}O$	**Citral***	1222	1224	1608	3,0
$C_{10}H_{16}O$	Geranial	1270	1252	1650	3,0
$C_{10}H_{18}O$	Citronellal	1132	1134	1458	3,1
Monoterpene acids					
$C_{10}H_{16}O_2$	Geranic acid	1333	1332	1758	2,8
Sesquiterpenes					
$C_{15}H_{24}$	β-Copaene	1418	1446	1938	2,8
$C_{15}H_{24}$	α-Muurolene	1480	1449	1944	2,6
$C_{15}H_{20}$	α-Calacorene	1517	1465	1980	2,7
$C_{15}H_{22}$	trans-Calamenene	1450	1457	1962	2,7
$C_{15}H_{20}$	α-Calacorene	1517	1465	1980	2,7
$C_{15}H_{24}$	Aromadendrene	1447	1484	2022	2,6
Sesquiterpenoids					
$C_{15}H_{24}$	β-Farnesene	1445	1440	1950	2,6

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cherry silver berry
28 terpenes

Formula	Name	$LRI_{lit.}$	$LRI_{calc.}$	^1Dt_R[s]	^2Dt_R[s]
Monoterpene hydrocarbons					
$C_{10}H_{16}$	**α-Pinene***	937	**937**	1074	2,7
$C_{10}H_{16}$	**R-(+)-Limonene***	1026	**1025**	1254	2,9
$C_{10}H_{18}$	**β-Myrcene***	985	**985**	1176	2,8
$C_{10}H_{14}$	**p-Cymene***	1036	**1078**	1356	3,2
$C_{10}H_{16}$	**β-trans-Ocimene***	1045	**1041**	1284	2,8
$C_{10}H_{16}$	**α-Phellandrene***	1003	**1003**	1212	2,8
$C_{10}H_{16}$	**Terpinolene***	1084	**1084**	1368	3,0
$C_{10}H_{16}$	**γ-Terpinene***	1051	**1053**	1308	2,9
$C_{10}H_{16}$	α-Thujene	921	**923**	1176	2,7
$C_{10}H_{16}$	β-Thujene	968	**951**	1104	2,8
$C_{10}H_{16}$	Cyclohexene, 1,5,5-trimethyl-3-methylen-	992	**991**	1188	2,8
$C_{10}H_{16}O$	**β-Cyclocitral***	1215	**1208**	1584	3,2
Monoterpenols					
$C_{10}H_{20}O$	**(-)-Menhtol***	1150.4	**1169**	1518	3,0
$C_{10}H_{18}O$	**4-Terpineol**	1172	**1172**	1524	3,2
$C_{10}H_{18}O$	**(-)-Linalool***	1087	**1087.5**	1374	3,0
$C_{10}H_{18}O$	**α-Terpineol***	1182	**1183**	1542	3,3
Monoterpene ketones					
$C_{10}H_{16}O$	trans-3-Pinanone, (E)-Pinocamphone	1143	**1148**	1482	3,4
$C_{10}H_{16}O$	**(-)-Camphor***	1127	**1131**	1452	3,6
$C_{10}H_{14}O$	(-)-Carvone	1212	**1228**	1614	3,2

Formula	Name	$LRI_{lit.}$	$LRI_{calc.}$	^1Dt_R[s]	^2Dt_R[s]
Monoterpene oxides					
$C_{10}H_{12}O_2$	Linalool oxide	1078	**1078**	1356	3,0
$C_{10}H_{18}O$	trans-Rose oxide	1115	**1117**	1428	3,0
Monoterpene aldehydes					
$C_{10}H_{16}O$	**Citral***	1222	**1224**	1608	3,0
$C_{10}H_{16}O$	Geranial	1270	**1252**	1650	3,0
Sesquiterpenes					
$C_{15}H_{22}$	β-Vatirenene	1452	**1441**	1926	2,6
$C_{15}H_{20}$	α-Calacorene	1517	**1465**	1980	2,7
$C_{15}H_{24}$	Aristolene	1423	**1389**	1824	2,5
Sesquiterpenoids					
$C_{15}H_{24}$	α-Farnesene	1490	**1446**	1938	2,5
Sesquiterpen oxides					
$C_{15}H_{24}O$	Leden oxide-(II)	1490	**1481**	2016	2,7

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scarlet hawthorn
30 terpenes

Formula	Name	$LRI_{lit.}$	$LRI_{calc.}$	^1Dt_R[s]	^2Dt_R[s]
Monoterpene hydrocarbons					
$C_{10}H_{16}$	**β-Pinene***	981	**980**	1170	2,7
$C_{10}H_{16}$	**α-Pinene***	937	**937**	1074	2,7
$C_{10}H_{14}$	1,3,8-p-Menthatriene	1105	**1103**	1404	3,1
$C_{10}H_{18}$	**β-Myrcene***	985	**985**	1176	2,8
$C_{10}H_{14}$	**p-Cymene***	1036	**1078**	1356	3,2
$C_{10}H_{16}$	**α-Phellandrene***	1003	**1003**	1212	2,8
$C_{10}H_{16}$	Cyclohexene, 1,5,5-trimethyl-3-methylen-	992	**991**	1188	2,8
$C_{10}H_{16}$	(+)-4-Carene, 2-(acetylmethyl)-	1382	**1353**	1782	2,8
$C_{10}H_{16}O$	**β-Cyclocitral***	1215	**1208**	1584	3,2
Monoterpenols					
$C_{10}H_{18}O$	**Eucalyptol ***	1030	**1028**	1260	3,0
$C_{10}H_{18}O$	4-Terpineol	1172	**1172**	1524	3,2
$C_{10}H_{18}O$	**(-)-Linalool***	1087	**1087.5**	1374	3,0
$C_{10}H_{18}O$	**α-Terpineol***	1182	**1183**	1542	3,3
Monoterpene ketones					
$C_{13}H_{20}O$	α-Ionone	1428	**1411**	1860	2,7
$C_{10}H_{16}O$	trans-3-Pinanone, (E)-Pinocamphone	1143	**1148**	1482	3,4
$C_{10}H_{16}O$	**(-)-Camphor***	1127	**1131**	1452	3,6
$C_{10}H_{14}O$	(-)-Carvone	1212	**1228**	1614	3,2
$C_{10}H_{14}O$	Pinocarvone	1150	**1148**	1482	3,6
$C_{10}H_{16}O$	**(-)-Fenchone***	1080	**1075**	1350	3,3

Formula	Name	$LRI_{lit.}$	$LRI_{calc.}$	^1Dt_R[s]	^2Dt_R[s]
Monoterpene oxides					
$C_{10}H_{18}O$	trans-Rose oxide	1115	**1117**	1428	3,0
Monoterpene phenols					
$C_{10}H_{14}O$	Thymol	1267	**1272**	1680	3,0
Monoterpene aldehydes					
$C_{10}H_{16}O$	Carvenone	1277	**1244**	1638	3,2
Sesquiterpenes					
$C_{15}H_{20}$	α-Calacorene	1517	**1465**	1980	2,7
$C_{15}H_{24}$	trans-α-Bergamotene	1405	**1394**	1830	2,5
$C_{15}H_{20}$	α-Calacorene	1517	**1465**	1980	2,7
Sesquiterpenoids					
$C_{15}H_{24}$	β-Caryophyllene	1412	**1416**	1872	2,6
$C_{15}H_{24}$	β-Ylangene	1442	**1441**	1926	2,5
$C_{15}H_{24}$	α-Farnesene	1490	**1446**	1938	2,5
Sesquiterpen oxides					
$C_{15}H_{24}O$	Caryophyllene oxide	1547	**1505**	2016	2,7
Hemiterpenes					
$C_{10}H_{16}$	Santolina triene	1089	**1084**	1368	3,0

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11 identical terpenes were determined in all analyzed fruits

α-Calacorene
(-)-Camphor
p-Cymene
β-Cyclocitral
Cyclohexene, 1,5,5-trimethyl-3-methylen-
(-)-Linalool
β-Myrcene
α-Phellandrene
α-Pinene
trans-3-Pineone
α-Terpineol

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Results

A total of **80** terpene compounds were separated and identified, including:

- **18** monoterpene hydrocarbons,
- **23** monoterpenols,
- **8** monoterpene ketones,
- **3** monoterpene oxides,
- **1** monoterpene phenols,
- **5** monoterpene aldehydes,
- **1** monoterpene acids,
- **10** sesquiterpenes,
- **8** sesquiterpenoids,
- **2** sesquiterpen oxides,
- **1** hemiterpenes.

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Results

Terpene profile:

- **63** terpenes were identified in **cape gooseberry**,
- **39** terpenes were identified in **crabapple**,
- **28** terpenes were identified in **cherry silver berry**,
- **30** terpenes were identified in **scarlet hawthorn**.

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Results

Cape gooseberry

- GC-MS (SLB-5ms column, *n*-alcanes standard solution) = **138** compounds
- GC-MS (Supelcowax-10, *n*-alcanes standard solution) = **49** compounds
- GC-MS (Supelcowax-10, FAMEs standard solution) = **52** compounds
- GC-MS (Supelcowax-10, FAEEs standard solution) = **21** compounds

with similarity value>70% and ΔLRT ±10

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Results

Cape gooseberry

- GCxGC with cryogenic modulator = **238** compounds
- GCxGC with flow modulator = **172** compounds

with similarity value>70% and LRI tolerance of ± 20

using *n*-alkanes standard solution

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Summary

- **HS-SPME** technique was useful in classification trials of these fruits to the group of superfruits
- **80 terpenes** was separated and identified
- a large group of identified terpenes is characterized in literature by many **health properties and flavors**

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Summary

- further research on selected **superfruits** will allow to a better characterization of these fruits enabling define:

  - the **ripeness**,

  - **degree of processing**,

  - **quality**,

  - **geographical and botanical origin**

  by using a variety of techniques for enrichment and isolation of the analytes

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Acknowledgements

- This research was financially supported by the Polish National Science Centre for science in 2013-2016 (Grants No DEC-2012/07/N/ST4/00629)
- The authors wish also to thank LECO Poland for financial and technical support
- This research work was supported by the European Social Fund, the State Budget and the Pomorskie Voivodeship within the system project of the Pomorskie Voivodeship "InnoDoktorant – Scholarships for PhD students, VIth edition"

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Thank you for your attention!!

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