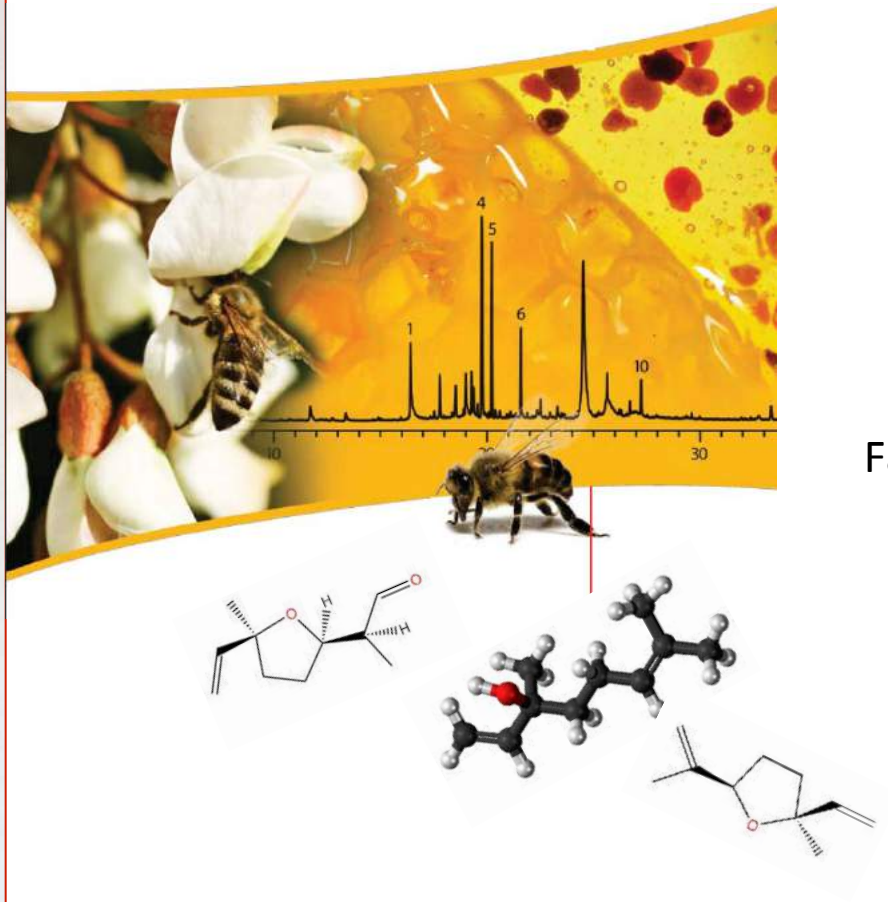
Application of HS-SPME/GC-MS for characterisation of Satsuma mandarin (*Citrus unshiu* Marc.) honey: nectar / honey-sac / honey headspace volatiles



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1. CONCEPTS OF HONEY AUTHENTIFICATION

(EU Council directive 2001/110/EC):

- melissopalynological analysis
- physico-chemical routine methods
- phytochemical fingerprinting methods:
  - honey
  - honey/nectar/plant
  - honey/honey sac

honey constituents

plant constituents

phytochemicals transfer and/or transformation by the bees; addition of specific compounds

3. HONEY CHEMICAL COMPOSITION

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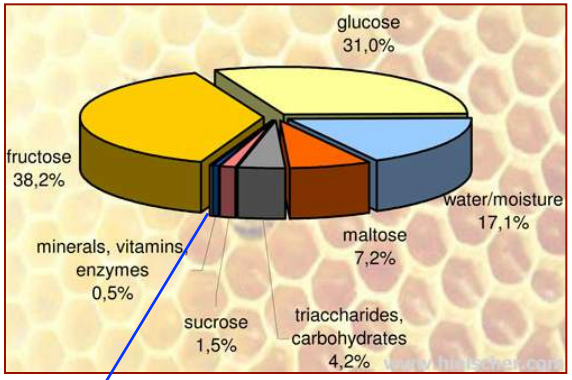


others 3.2%

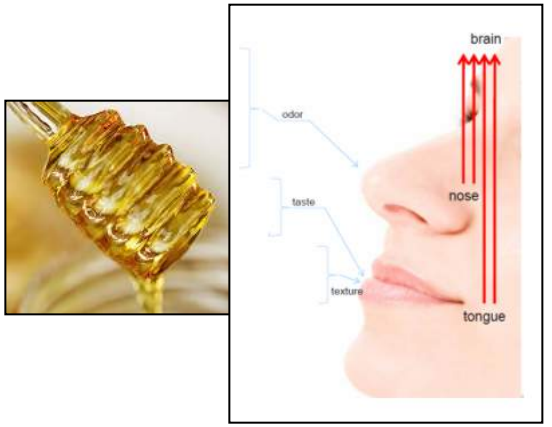
POTENTIAL BIOMARKERS OF HONEY BOTANICAL ORIGIN

- specific
- nonspecific

- phytochemicals
- pollen

- volatiles
  - terpenes
  - norisoprenoids
  - other volatiles
  - benzene derivatives
- phenols
  - flavonoids
  - non-flavonoid phenolic compounds
- carbohydrates
- N containing compounds
  - amino acids
  - non-amino acids
- other minor compounds
  - microelements
  - miscellaneous

Volatile organic compounds (VOCs): characteristic of different honey types with potential for honey discrimination (specific chemical profiles and markers of botanical origin), potential non-enzymatic antioxidants and compounds with non-peroxide antimicrobial activity



Origin of VOCs in honey:

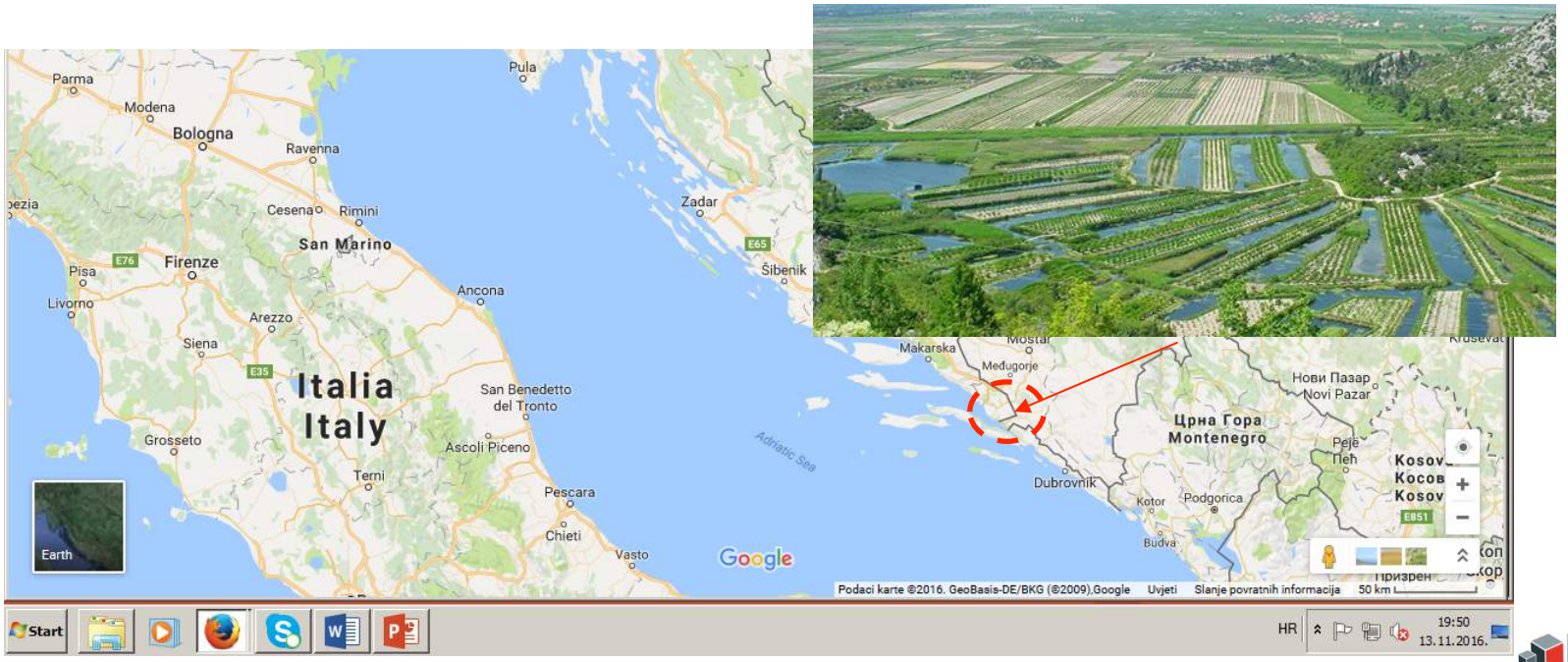
- transfer from the plant (phytochemicals)
- bees transformation of plant compounds
- bees compounds introduced in the honey
- compounds derived from thermal treatment of honey or prolonged storage
- environmental contaminants and others

4. ORIGIN OF THE SAMPLES

Neretva valley, Opuzen area, Croatia:

- around 2.5 million mandarin trees have been planted in the Opuzen area (ca. 2500 ha) providing a good nectar source for unifloral honey production

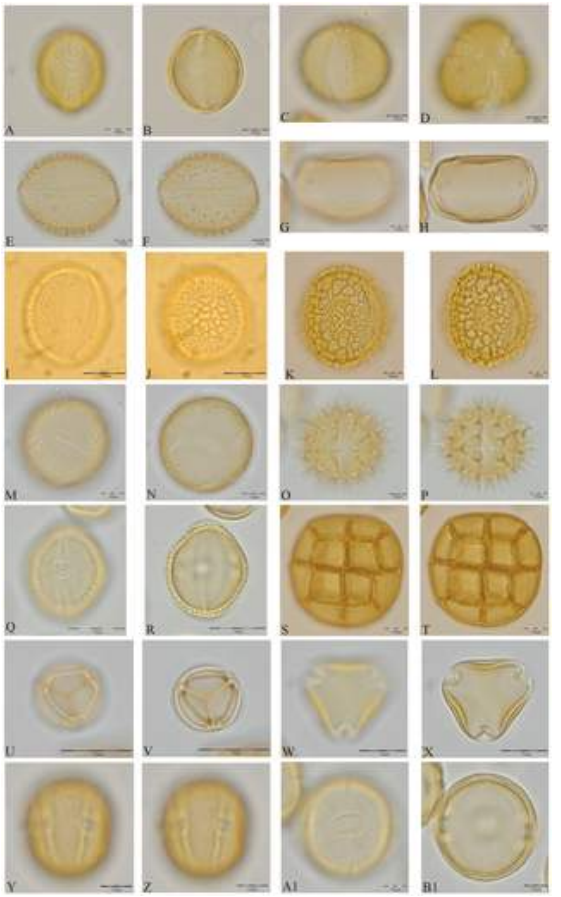
- 90% of Citrus orchards were Satsuma mandarins (*Citrus unshiu* Marc.), varieties *Zorica*, *Chahara*, *Kawano Wase*, and *Okitsu*, while others were clementine (*C. clementina* Hort. ex Tan.), sweet orange (*C. sinensis*), grapefruit (*C. paradisi*) and lemon (*C. limon*)



- melissopalynological analysis has been accepted as the reference method to authenticate honey botanical origin

- however, pollen analysis is considered of little value for the Citrus genus as it is one of several honey types with underrepresented pollen; accordingly, *C. unshiu* honey characterization is difficult with underrepresented pollen due to the specific plant physiology of particular mandarin cultivars (aborted anthers, sterile pollen grains, or partenocarpy)

- therefore, there is a need for detailed chemical characterization of *C. unshiu* honey and present research is focused on its volatile organic compounds (VOCs)

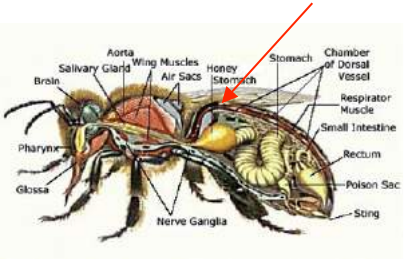


- the nectars (1.5 mL) from the varieties *Zorica*, *Chahara*, *Kawano Wase*, and *Okitsu* were collected with microcapillary glass tubes from flowers

- during *C. unshiu* honey flow, a part of the returning foragers were collected; the bees were frozen in the field by liquid nitrogen and were stored in a deep-freezer until their honey-sac contents were investigated

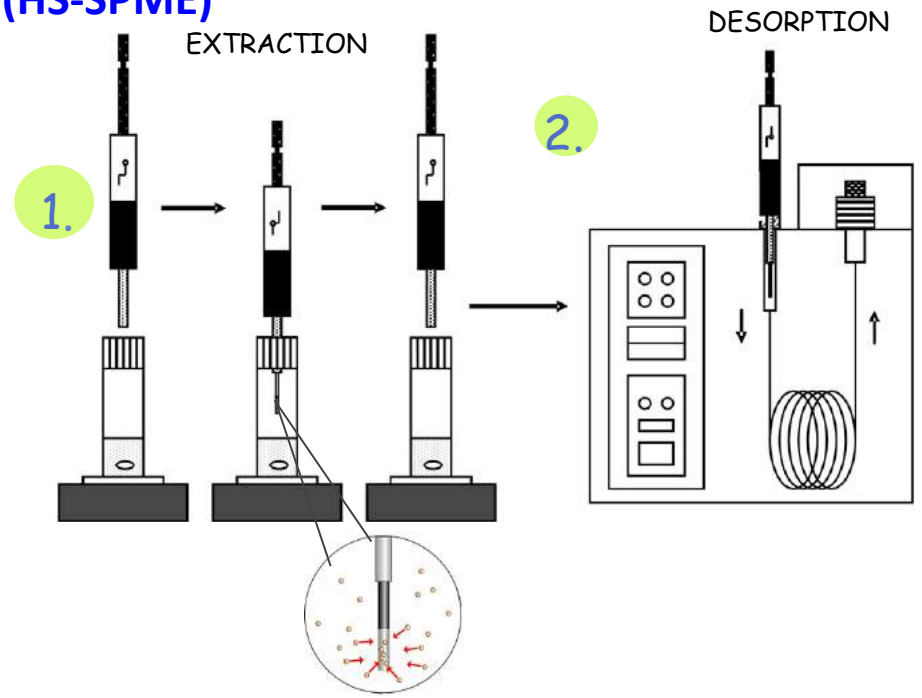
- after thawing, the abdomen of 100 bees was dissected by peeling off the tergit with forceps in order to expose the honey sac; the honey sacs were removed and frozen

- after freezing, the entire content of the honey-sacs was pooled and put in a glass vial (5 mL) at 4◦C until the volatiles were isolated



5. Headspace solid-phase microextraction (HS-SPME)

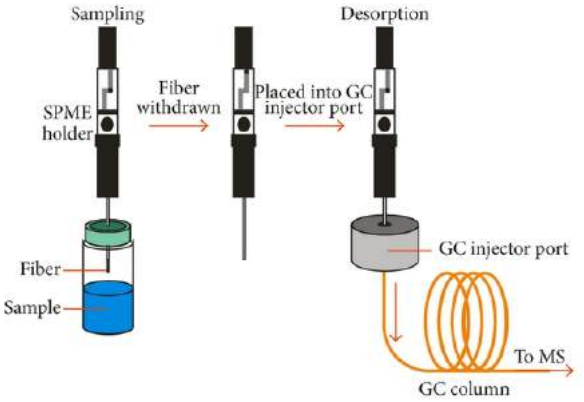
- short, thin solid rod of fused silica, coated with absorbent polymer is used
- equilibrium partitioning of the compounds between the coating fiber and the headspace
- the main advantages of SPME: simplicity, high sensitivity, small sample volume, and lower cost



Stationary phase	Recommended use
PDMS: polydimethylsiloxane	for volatiles (non-polar fibre)
PA: polyacrilate	for polar semivolatile compounds (polar fibre)
CW/DBV: carbowax/divinylbenzene	for alcohols and volatiles (polar fibre)
PDMS/DVB: polydimethylsiloxane/divinylbenzene	for volatile compounds, amines and nitroaromatics (non-polar fibre)
DVB/CAR/PDMS: divinylbenzene/carboxene/polydimethylsiloxane	for odours

- the headspace extraction was performed using a manual SPME holder using two fibres: divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS) and polydimethylsiloxane/divinylbenzene (PDMS/DVB)

- for HS-SPME, the nectars (1 mL) were placed separately in 5 mL glass vials and hermetically sealed with PTFE/silicone septa; the content of honey-sacs was put as described above in 5 mL glass vials; the honey/saturated water solution (5 mL, 1:1 (v/v); saturated with NaCl) of each honey sample was placed in a 15 mL glass vial and hermetically seale



- the vials were maintained in a water bath at 60◦C during equilibration (15 min) and HS-SPME (45 min) under constant stirring (1000 rpm) with a magnetic stirrer; after sampling, the SPME fibre was withdrawn into the needle, removed from the vial, and inserted into the injector (250◦C) of the GC-FID and GC-MS for 6 min where the extracted volatiles were thermally desorbed to the GC column

Table 1. The headspace chemical composition of the honey-sac (HoS) and nectars (NE) of different *C. unshiu* varieties determined by HS-SPME/GC-MS/FID analysis.

No.	Compound	RI	Area (%) Fibre PDMS/DVB					Area (%) Fibre DVB/CAR/PDMS				
			HoS	NE_A	NE_B	NE_C	NE_D	HoS	NE_A	NE_B	NE_C	NE_D
1	Acetic acid	<900	1.8	0.4	0.3	-	-	4.9	-	-	-	0.9
2	3-Hydroxybutan-2-one	<900	-	0.2	0.3	-	-	-	-	-	-	-
3	Pentan-1-ol	<900	1.3	-	-	-	-	1.3	-	-	-	-
4	Hexanal	<900	-	0.1	0.1	0.5	0.7	-	-	-	0.3	-
5	(Z)-Hex-3-en-1-ol	<900	-	2.9	1.7	0.2	-	-	0.3	0.1	-	-
6	Hexan-1-ol	<900	-	4.9	5.2	-	-	-	0.5	0.3	-	-
7	Heptan-2-one	<900	5.5	0.7	-	-	-	-	0.2	-	-	-
8	Heptan-2-ol	<900	7.8	-	-	-	-	3.3	-	-	-	-
9	α-Pinene	940	-	0.1	0.1	-	-	-	0.1	0.3	-	-
10	Benzaldehyde	965	1.0	0.1	0.3	2.2	3.6	2.7	-	-	0.6	-
11	Hexanoic acid	980	-	-	-	-	-	0.9	-	0.5	-	-
12	β-Pinene	982	-	0.1	0.1	-	0.4	-	0.5	-	-	-
13	6-Methylhept-5-en-2-one	989	-	0.6	0.8	0.2	-	-	0.5	0.3	0.6	-
14	Octan-2-one	993	-	1.1	0.4	-	-	-	1.5	-	-	-
15	β-Myrcene	994	-	-	-	-	-	-	-	0.4	-	-
16	6-Methylhept-5-en-2-ol	995	-	-	0.4	-	-	-	-	0.0	-	-
17	α-Terpinene	1022	-	0.1	0.1	-	-	-	-	0.4	-	-
18	*p*-Cymene	1029	4.0	3.0	2.1	-	1.1	-	3.6	3.7	0.3	-
19	Limonene	1034	-	1.3	0.9	-	-	-	1.0	1.5	-	-
20	1,8-Cineole	1037	1.9	2.8	1.3	0.3	0.4	3.6	3.1	1.5	0.6	-
21	Benzyl alcohol	1038	2.9	-	-	-	-	3.6	-	-	-	-
22	*cis*-β-Ocymene	1042	-	-	-	-	-	-	0.2	0.1	-	-
23	Phenylacetaldehyde	1048	0.5	-	0.7	5.5	5.6	2.0	-	0.3	3.2	7.0
24	*trans*-β-Ocymene	1052	-	0.7	0.8	-	0.7	-	2.4	4.1	0.0	-
25	γ-Terpinene	1064	1.1	4.4	2.8	-	0.9	-	6.6	8.1	0.3	-
26	Octan-1-ol	1074	-	-	1.1	-	-	-	0.5	0.5	-	-
27	α-Terpinolene	1092	-	0.4	0.1	-	-	-	0.3	1.1	-	-
28	Nonan-2-one	1094	-	0.4	-	-	-	-	0.8	-	-	-
29	Methyl benzoate	1098	-	0.4	-	0.2	-	-	0.3	-	-	-
30	Linalool	1102	15.2	21.7	21.3	1.2	1.6	3.8	21.1	10.4	5.3	6.8
31	2-Phenylethanol	1118	2.9	4.1	2.9	1.3	2.7	6.7	2.8	1.2	0.6	1.2
32	Methyl octanoate	1128	-	-	-	-	-	-	0.2	0.4	-	-
33	Phenylacetonitrile	1143	11.0	-	1.9	7.2	7.3	10.4	-	1.2	5.0	6.3
34	Isopulegol	1151	-	-	5.2	-	-	-	-	1.8	-	-
35	Isomenthone	1159	-	-	0.3	-	-	-	-	0.0	-	-
36	Nonan-1-ol	1178	-	0.2	-	-	-	-	0.8	-	-	-
37	Terpinen-4-ol	1181	4.1	5.9	5.8	0.3	-	2.0	3.6	1.5	0.9	0.7
38	*p*-Cymen-8-ol	1189	-	-	-	-	-	-	0.2	-	-	-
39	α-Terpineol	1194	10.2	16.0	11.4	3.0	2.4	9.3	9.0	4.1	4.1	6.1
40	2-Aminobenzaldehyde *	1218	-	-	0.1	0.5	1.1	-	0.2	0.1	0.3	-
41	Methyl nonanoate	1228	-	-	-	-	-	-	0.5	0.8	0.6	-

Table 1. *Cont.*

No.	Compound	RI	Area (%) Fibre PDMS/DVB					Area (%) Fibre DVB/CAR/PDMS				
			HoS	NE_A	NE_B	NE_C	NE_D	HoS	NE_A	NE_B	NE_C	NE_D
42	Piperitone	1253	-	0.1	0.1	-	-	-	0.1	0.0	0.0	-
43	Geraniol	1260	-	0.5	0.5	-	-	-	0.7	0.8	1.2	-
44	1*H*-Indole	1295	7.9	7.3	11.5	52.5	52.3	8.9	12.2	16.5	47.4	39.6
45	Methyl decanoate	1328	-	-	-	-	-	-	-	0.4	-	-
46	Methyl anthranilate	1344	7.7	6.1	3.9	8.5	3.0	19.8	5.6	5.6	9.1	9.1
47	β-Elemene	1394	-	0.5	-	-	1.1	-	0.9	1.5	-	-
48	*cis*-Jasmone	1399	5.1	3.6	2.8	3.0	1.1	6.9	5.1	6.0	7.9	2.8
49	*trans*-Caryophyllene	1421	-	1.3	0.8	-	2.2	-	1.5	2.6	-	-
51	(*E*,*Z*)-α-Farnesene	1496	-	-	-	-	-	-	0.5	0.0	-	-
52	(*E*,*E*)-α-Farnesene	1503	-	0.4	0.1	-	-	-	2.0	2.6	-	0.7
53	Methyl dodecanoate	1523	-	-	-	-	-	-	0.2	-	-	0.9
54	Caryophyllene oxide	1584	-	-	-	-	-	-	-	0.7	-	-
55	Methyl tetradecanoate	1727	-	-	0.3	0.5	1.1	-	0.9	2.2	-	1.6
56	Methyl hexadecanoate	1929	-	0.4	0.9	1.3	2.7	-	-	5.5	-	6.1

HoS = honey-sac, NE_A = nectar *Kawano Wase*, NE_B = nectar *Chahara*, NE_C = nectar *Okitsu*, NE_D = nectar *Zorica*, RI = retention indices on HP-5MS column, * = tentatively identified.

- the major headspace compounds were N-containing compounds: 1H-indole (7.3%–52.5%; 12.2%–47.4%) and methyl anthranilate (3.0%–8.5%; 5.6%–19.8%)

- higher percentages of 1H-indole was found in NE *Okithu* and *Zorica* and methyl anthranilate in NE *Kawano Wase*

- those compounds derive from chorismate in the tryptophan biosynthetic pathway (Figure 1).

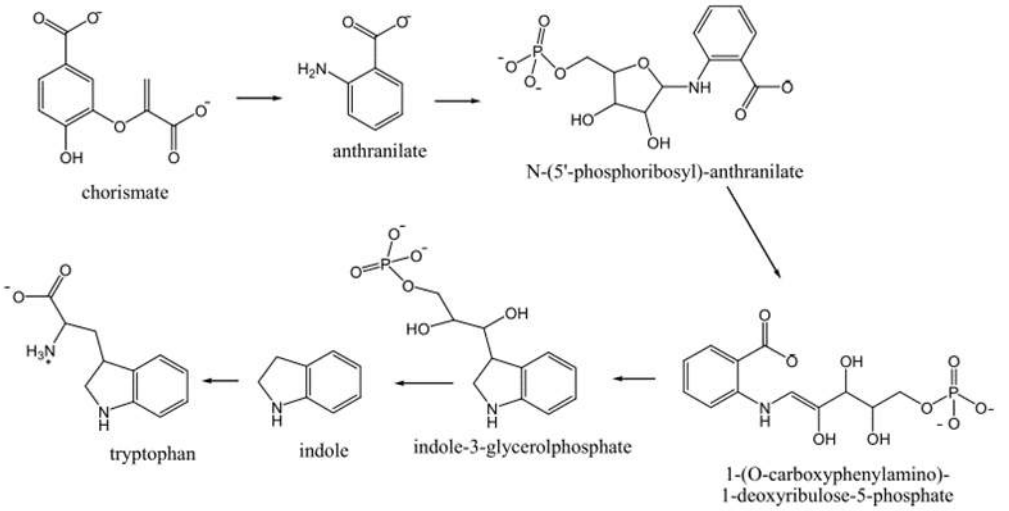
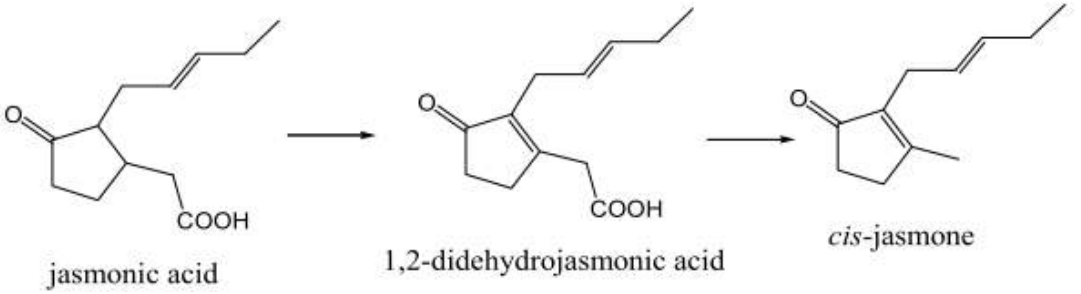


Figure 1. Tryptophan biosynthetic pathway.

PR-anthranilate transferase catalyzes phosphoribosyl moiety transfer from phospho-ribosylpyrophosphate to anthranilate. In the next step, PR-anthranilate isomerase rearranges PR-anthranilate to 1-(O-carboxyphenylamino)-1-deoxyribulose-5-phosphate. Indole-3-glycerolphosphate synthase next forms an indole ring.

- the major oxygenated monoterpenes in NE headspace were linalool (1.2%–21.7%; 5.3%–21.1%) and α-terpineol (2.4%–16.0%; 4.1%–9.3%); other abundant monoterpenes were terpinen-4-ol (0.0%–5.9%; 0.7%–3.6%), 1,8-cineole (0.4%–2.8%; 0.0%–3.7%) and γ-terpinene (0.0%–4.4%; 0.0%–8.1%); only a few sesquiterpenes were present with *trans*-caryophyllene (0.0%–2.2%; 0.0%–2.6%) as the major one

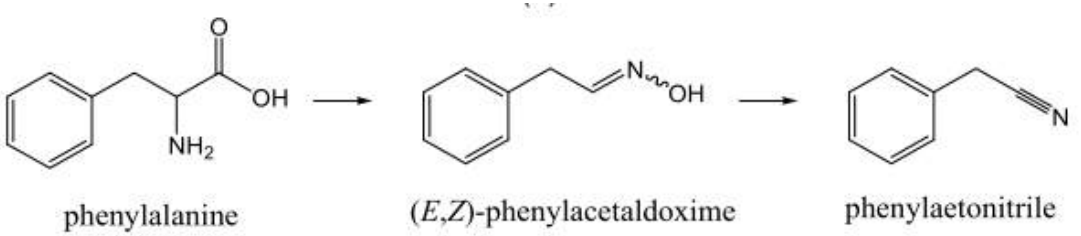
- *cis*-jasmone (*cis*-3-methyl-2-(2-pentenyl)-cyclopent-2-en-1-one) was found (1.1%–3.6%; 2.8%–7.9%) in all NE



jasmonic acid → 1,2-didehydrojasmonic acid → *cis*-jasmone

It is produced by the plants by an oxidative degradation of jasmonic acid (formed by lipoxygenase-catalyzed oxygenation of linolenic acid *via* 18-carbon cyclic fatty acid formed by the action of hydroperoxide cyclase, followed by reduction and β-oxidations), *via* 1,2-didehydrojasmonic acid; subsequent protonation of the carbonyl O-atom of 1,2-didehydrojasmonic acid is assumed to induce a Grob-type fragmentation of the molecule *cis*-jasmone.

- among benzene derivatives 2-phenylethanol (1.3%–4.1%; 0.6%–2.8%) and benzaldehyde (0.1%–3.6%; 0.0%–0.6%) were abundant; benzyl alcohol, phenylacetaldehyde (more abundant in NE *Okitsu* and *Zorica*) and methyl benzoate were also found; phenylacetonitrile (0.0%–7.3%; 0.0%–6.3%) formation has been found in several secondary metabolic pathways initiating from phenylalanine in the plants



phenylalanine (*E*,*Z*)-phenylacetaldoxime phenylaetonitrile

Phenylalanine is first converted to (*E*,*Z*)-phenylacetaldoxime, which is then transformed to 2-hydroxy-2-phenylacetonitrile, probably via phenylacetonitrile formation as the intermediate.

- NE headspace also contained lower aliphatic compounds up to C10, most probably derived from fatty acid degradation: alcohols (e.g., (*Z*)-hex-3-en-1-ol, pentan-1-ol or hexan-1-ol), ketones (e.g., heptan-2-one or octan-2-one), acids (acetic and hexanoic), and methyl esters (octanoate, nonanoate and decanoate)

Table 3. The hedaspace composition of *C. unshiu* honey (n = 12) determined by HS-SPME, followed GC-FID and GC-MS analysis.

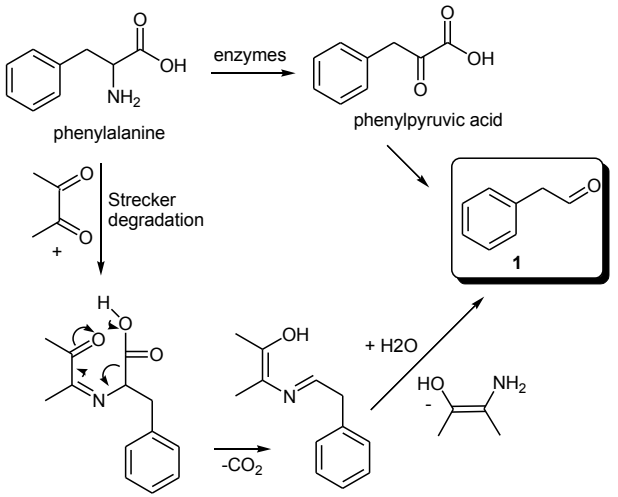
No.	Compound	RI	Area (%) Fibre PDMS/DVB				Area (%) Fibre DVB/CAR/PDMS			
			Min.	Max.	Av.	SD.	Min.	Max.	Av.	SD.
1	Ethanol	<900	0.0	6.7	2.28	2.57	0.0	1.3	0.26	0.58
2	Acetic acid	<900	0.0	8.1	3.22	3.40	0.0	2.6	0.82	1.03
3	Butanal	<900	0.0	2.3	1.02	1.09	0.0	2.8	0.58	1.24
4	Ethyl acetate	<900	0.0	2.4	0.88	1.21	0.0	0.0	0.00	0.00
5	3-Methylbutanal	<900	0.0	0.9	0.42	0.45	0.0	0.0	0.00	0.00
6	Butan-1-ol	<900	0.0	3.3	1.26	1.73	0.0	0.0	0.00	0.00
7	Pentanal	<900	0.0	0.0	0.00	0.00	0.0	0.7	0.14	0.31
8	3-Hydroxybutan-2-one	<900	0.0	0.0	0.00	0.00	0.0	0.0	0.00	0.00
9	Ethylisocyanide *	<900	0.0	0.3	0.06	0.13	0.0	0.0	0.00	0.00
10	3-Methylbutanenitrile *	<900	0.0	1.4	0.28	0.63	0.0	0.0	0.00	0.00
11	Butanoic acid	<900	0.0	0.7	0.16	0.30	0.0	0.0	0.00	0.00
12	3-Methylbutan-1-ol	<900	0.0	1.9	0.38	0.85	0.0	0.0	0.00	0.00
13	Octane	<900	0.0	1.5	0.46	0.68	0.0	2.7	0.94	1.03
14	Hexanal	<900	0.0	0.7	0.22	0.32	0.0	1.3	0.33	0.65
15	Furfural	<900	0.5	1.9	1.18	0.51	0.0	0.6	0.24	0.33
16	Dihydro-2-methyl-3(2*H*)-furanone	<900	0.0	0.9	0.18	0.40	0.0	0.0	0.00	0.00
17	Isoamylacetate	<900	0.0	1.9	0.38	0.85	0.0	0.0	0.00	0.00
18	Nonane	900	0.0	0.0	0.00	0.00	0.0	1.1	0.24	0.48
19	Heptanal	902	0.0	0.0	0.00	0.00	0.0	0.3	0.10	0.14
20	Benzaldehyde	965	5.8	9.8	7.18	1.86	3.3	6.6	5.06	1.46
21	Hexanoic acid	980	0.0	0.8	0.26	0.37	0.0	0.0	0.00	0.00
22	6-Methylhept-5-en-2-one	989	0.0	0.0	0.00	0.00	0.0	0.9	0.30	0.39
23	Ethyl hexanoate	1001	0.0	0.4	0.08	0.18	0.0	0.0	0.00	0.00
24	Octanal	1004	0.0	0.0	0.00	0.00	0.4	0.9	0.58	0.22
25	*p*-Cymene	1029	0.0	0.0	0.00	0.00	0.0	0.4	0.22	0.16
26	Benzyl alcohol	1038	0.0	2.8	1.32	1.02	0.0	0.0	0.00	0.00
27	Phenylacetaldehyde	1048	34.4	47.2	41.92	5.85	38.3	49.1	43.36	4.35
28	*cis*-Linalool oxide	1075	3.0	11.5	5.48	3.49	0.0	4.1	1.94	1.51
29	*p*-Cymenyl	1095	0.0	0.8	0.36	0.38	0.7	2.7	1.66	0.81
30	Methyl benzoate	1098	0.0	6.2	1.24	2.77	0.0	14.5	2.90	6.48
31	Linalool	1102	0.0	2.2	1.30	0.87	0.0	4.5	3.18	1.83
32	Hotrienol	1105	1.4	2.6	1.96	0.56	1.2	2.3	1.66	0.42
33	Methyl octanoate	1128	2.0	4.9	3.12	1.45	2.3	5.3	3.88	1.38

Table 3. *Cont.*

No.	Compound	RI	Area (%) Fibre PDMS/DVB				Area (%) Fibre DVB/CAR/PDMS			
			Min.	Max.	Av.	SD.	Min.	Max.	Av.	SD.
34	Phenylacetonitrile	1143	2.7	9.9	5.44	3.12	3.4	10.2	6.62	3.04
35	Lilac aldehyde (isomer I) **	1173	1.0	5.6	2.82	2.09	1.5	7.2	3.80	2.69
36	Lilac aldehyde (isomer II) **	1178	0.0	0.5	0.10	0.22	0.0	0.8	0.16	0.36
37	Lilac aldehyde (isomer III) **	1188	0.0	0.0	0.00	0.00	0.0	0.4	0.16	0.22
38	Octanoic acid	1194	0.0	0.0	0.00	0.00	0.0	0.4	0.12	0.18
39	Dill ether	1198	0.0	0.7	0.14	0.31	0.0	0.0	0.00	0.00
40	α-Terpineol	1194	0.0	0.7	0.18	0.30	0.9	3.2	1.58	0.94
41	Decanal	1207	0.0	1.4	0.56	0.59	1.4	5.1	3.46	1.48
42	Methyl nonanoate	1217	0.0	1.0	0.20	0.45	0.0	0.0	0.00	0.00
43	8,9-Epoxy-*p*-menth-1-ene *	1218	0.0	0.0	0.00	0.00	0.0	4.6	2.82	1.80
44	*p*-Meth-9-en-1-al (isomer I) **	1221	0.0	1.1	0.22	0.49	0.0	0.0	0.00	0.00
45	*p*-Meth-9-en-1-al (isomer II) **	1257	0.0	0.5	0.10	0.22	0.0	0.5	0.10	0.22
46	4-Methoxybenzaldehyde	1276	0.0	0.3	0.06	0.13	0.0	0.7	0.22	0.32
47	3-Methyl-6-(1-methylethyl)-cyclohex-2-en-1-one	1258	0.0	0.0	0.00	0.00	0.0	1.2	0.52	0.52
48	Nonanoic acid	1272	2.1	3.3	2.86	0.50	2.3	4.9	3.60	1.25
49	1*H*-Indole	1295	0.0	0.0	0.00	0.00	0.0	0.8	0.40	0.38
50	Methyl anthranilate	1344	0.0	3.3	0.78	1.42	0.0	0.0	0.00	0.00
51	Methyl hexadecanoate	1929	0.0	0.5	0.00	0.22	0.0	0.0	0.00	0.00

Min. = minimal percentage, Max. = maximal percentage, Av. = average percentage, SD. = standard deviation, RI = retention indices on HP-5MS column, * = tentatively identified, ** = correct isomer is not identified.

- phenylacetaldehyde was dominant compound (34.4%–47.2%; 38.3%–49.1%) of the *C. unshiu* honey headspace, followed by benzaldehyde (5.8%–9.8%%; 3.3%–6.6%) and phenylacetonitrile (2.7%–9.9%; 3.4%–10.2%); phenylacetaldehyde was strikingly more abundant in comparison with the nectar headspace (HS-NE) and the headspace of the honey-sac (HS-HoS), indicating its formation during the honey ripening in the hive (generated from phenylalanine either by enzyme catalysis or by Strecker degradation)



- a high percentage of phenylacetaldehyde was found in the honey headspace of *Asphodelus microcarpus* Salz. et Viv.; phenylacetonitrile was present within percentage ranges similar to those seen in the HS-NE and HS-HoS, while benzaldehyde percentages were elevated; benzaldehyde was found to be the major volatile from the honey of cambara and willow, but also in lemon and orange honey; phenylacetonitrile was found in the headspace of dandelion and thyme honeys

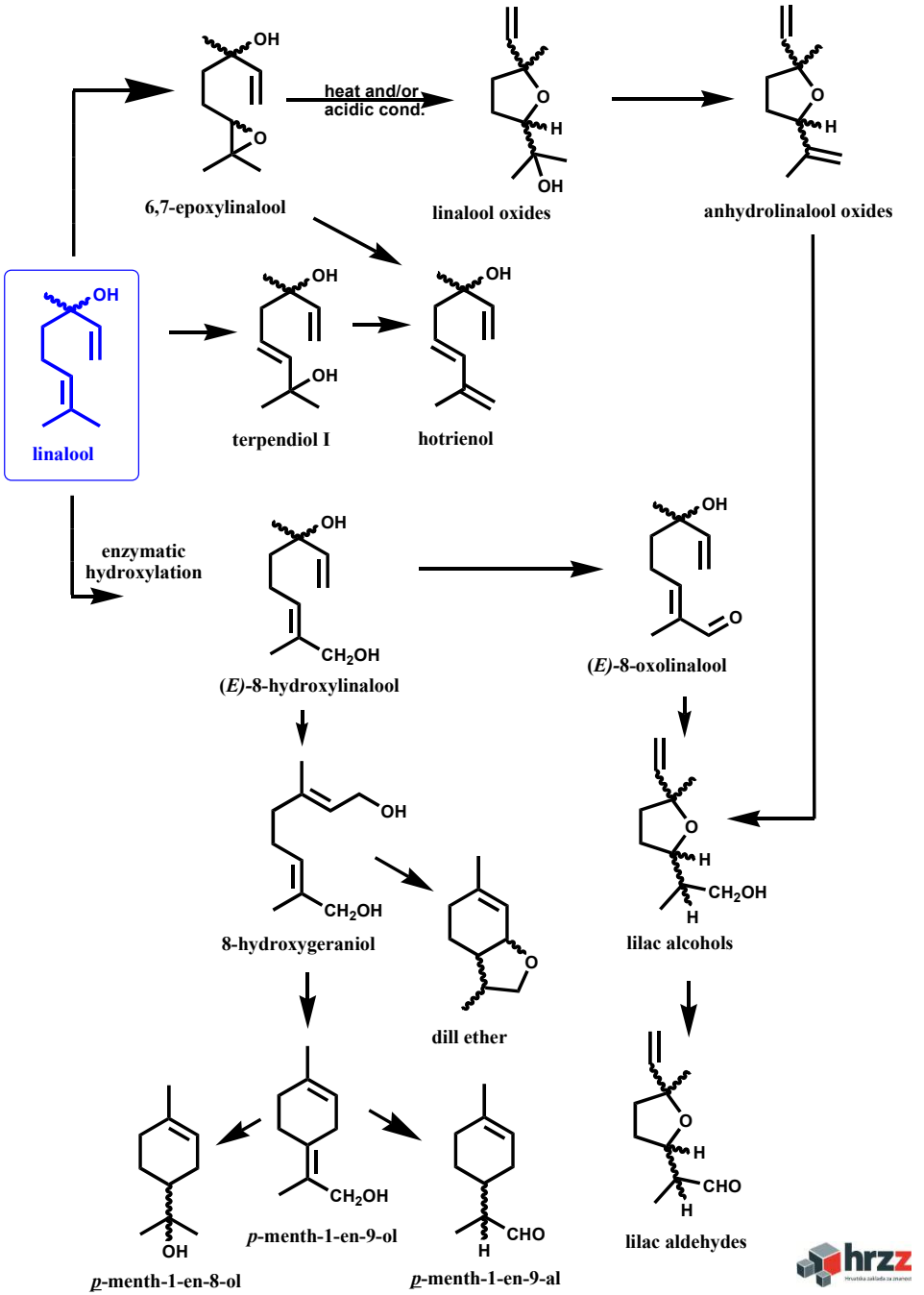
- linalool was present as a minor constituent (0.0%–2.2%; 0.0%–4.5%) in distinction to HS-NE and HS-HoS; an array of linalool derivatives were found, such as *cis*-linalool oxide (3.0%–11.5%; 0.0%–4.1%), hotrienol (1.4%–2.6%; 1.2%–2.3%), lilac aldehydes, dill ether or *p*-menth-9-en-1-al isomers, not present at all in HS-NE and HS-HoS; they were formed from linalool within the hive conditions.

- 1H-indole and methyl anthranilate were occasionally present, but not in the headspace of all honey samples, and with markedly lower percentages in comparison to HS-NE and HS-HoS

- among lower aliphatic compounds of the honey headspace, nonanoic acid was the most abundant (2.2%–3.3%; 2.3%–4.9%), but not found in HS-NE and HS-HoS

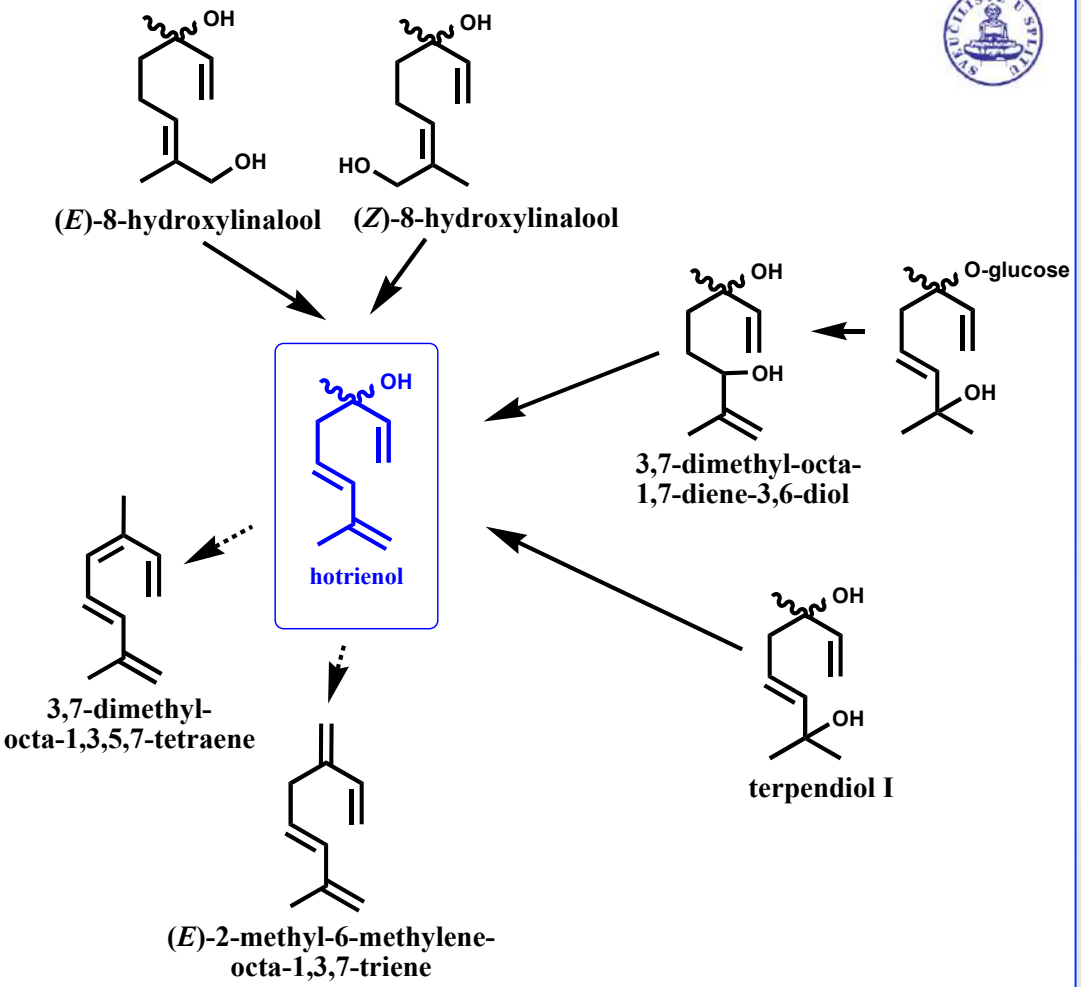
The bioconversion of linalool by honeybees under closed beehive conditions:

- the formation of furan/pyran linalool oxides and terpendiol I was catalysed by the enzymes secreted by the bees
- the formation of lilac aldehydes, *cis*- and *trans*-anhydrolinalool oxides, (*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienal (*E*)-8-hydroxylinalool and (*Z*)-8-hydroxylinalool require plant-derived enzyme
- the hive acidic conditions do not lead to the formation of typical linalool derivatives



Alissandrakis et al., *Eur. Food Res. Technol.*, 231 (2010) 21.

- hotrienol is particularly labile compound among terpenes known to be a thermally generated product, but there are findings that support its natural occurrence in non-thermally treated honey



Eucryphia lucida Baill.

- hotrienol is the principal component detected in leatherwood (*Eucryphia lucida* Baill.) honey headspace, while 2,6-dimethylocta-3,7-diene-2,6-diol and hotrienol were major compounds of the extracts; the diol has also been detected in the nectar

Rowland et al., *J. Agric. Food Chem.*, 43 (1995) 753.

6. Conclusions

- applied HS-SPME/GC-MS/FID methodology of monitoring nectar/honey-sac/ honey pathways of the headspace volatiles was successful for the characterisation of *C. unshiu* honey

- the major headspace compounds from all nectar varieties were linalool, α-terpineol, 1H-indole, methyl anthranilate and phenylacetonitrile

- the major headspace compounds of the honey-sac were linalool, α-terpineol, 1,8-cineole, 1H-indole, methyl anthranilate and *cis*-jasmone

- the honey headspace composition was significantly different in comparison to the nectars and the honey-sac content with respect to phenylacetaldehyde and linalool derivatives' abundances that appeared as the consequence of the hive conditions and the bee enzymes' activity

- *C. unshiu* honey traceability is determined by the following chemical markers: phenylacetaldehyde, phenylacetonitrile, linalool, and its derivatives

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