MONOTERPENI KAO KEMIJSKI MARKERI RAZLIČITIH VRSTA MEDA

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2. KEMIJSKI SASTAV MEDA

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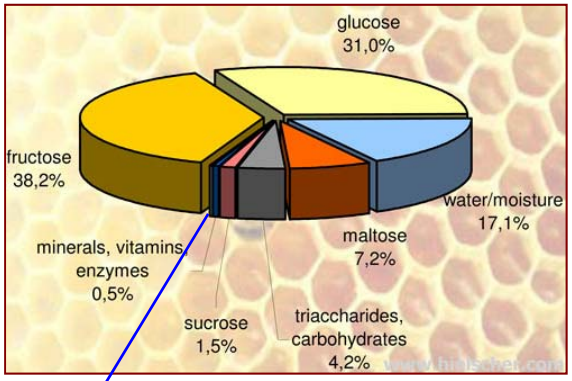


ostali spojevi

POTENCIJALNI BIOMARKERI BOTANIČKOG PORIJEKLA MEDA

- specifični
- nespecifični

- fitokemikalije
- polen

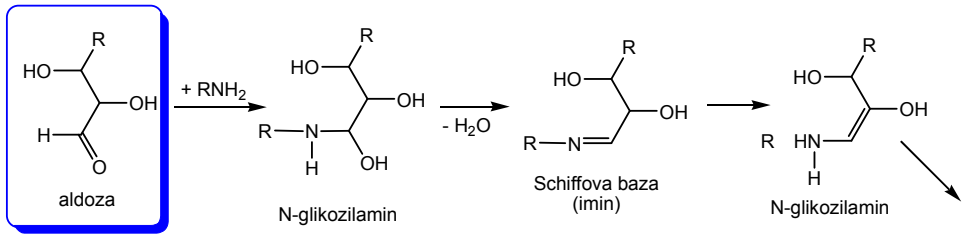
- isparljivi spojevi
  - terpeni
    - monoterpeni
  - norizoprenoidi
  - ostali isparljivi spojevi
  - derivati benzena
- fenoli
  - flavonoidi
  - ne-flavonoidni fenoli
- ugljikohidrati
- spojevi s N
  - aminokiseline
  - drugi spojevi s N
- ostali spojevi
  - mikroelementi
  - drugi spojevi

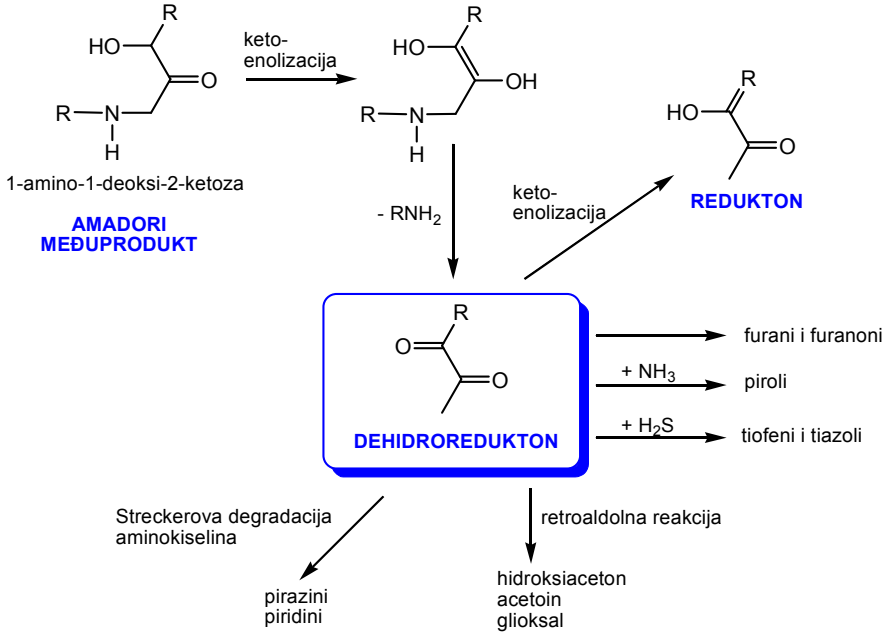
3. METODE EKSTRAKCIJE ISPARLJIVIH SPOJEVA MEDA

Odabir metode:

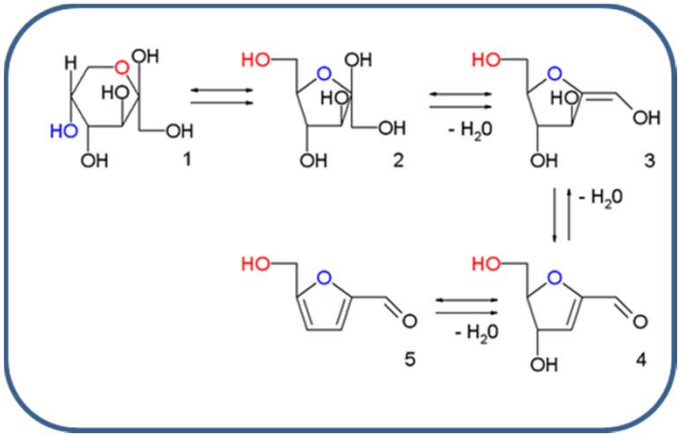
- zagrijavanje uzorka meda → ARTEFAKTI!

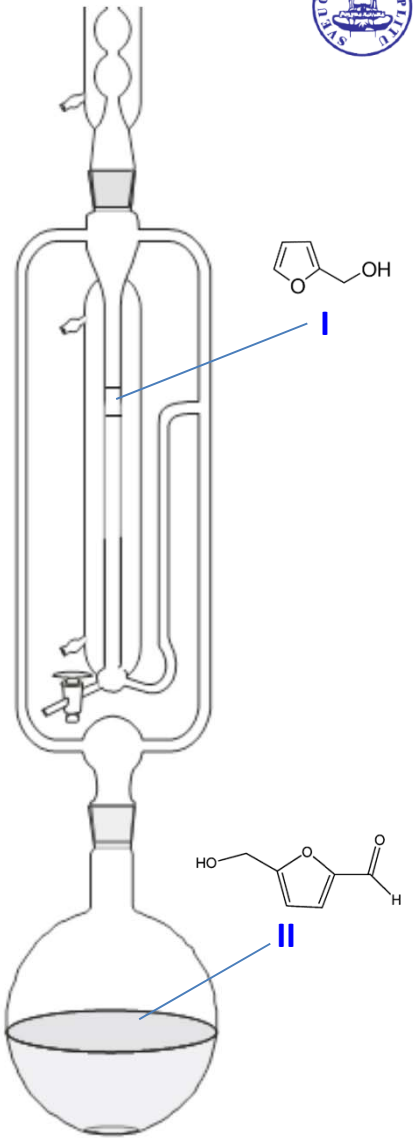
produkti Maillardovih reakcija:





***Rhamnus frangula* L.**



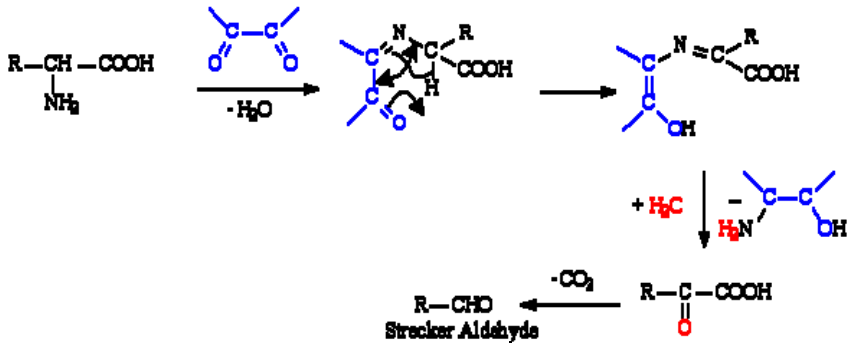


	Spoj	Min.	Max.	USE I	HD II	III
1.	Furfuril-alkohol	0,4	1,3	1,3	-	0,4
2.	5-HMF	-	-	-	15,6	5,0

I – destilat
II - CH_2Cl_2 ekstrakt vodene otopine meda (nakon destilacije)
III - CH_2Cl_2 ekstrakt izravno zagrijanog meda (bez dodatka H_2O)

I. Jerković i dr., *Chemistry of Natural Compounds* 51 (2016) 1174-1177

produkti Streckerovih degradacija:



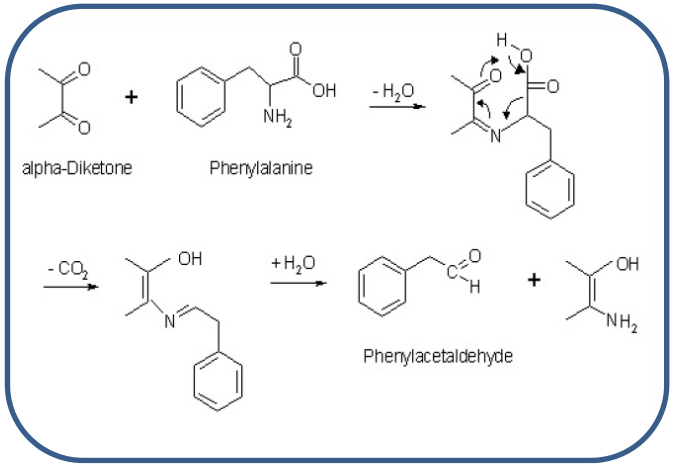


Table 1
Volatiles from *Pseudoacacia robinia* L.

	HD				USE			
	Min.	Max.	Avg.	δ	Min.	Max.	Avg.	δ
1 2-Methyldecane	–	–	–	–	1.6	3.1	2.23	0.78
2 Tridecane	–	–	–	–	0.6	2.9	1.50	1.23
3 Nonanal	–	–	–	–	0.6	0.7	0.60	0.10
4 Acetic acid	–	–	–	–	0.3	1.3	0.80	0.50
5 Decanal	–	–	–	–	0.4	0.5	0.43	0.06
6 *cis*-Linalool oxide	1.5	3.0	2.23	0.75	–	–	–	–
7 2-Furancarboxaldehyde (*Furf*	2.1	4.6	3.27	1.26	–	–	–	–
8 *trans*-Linalool oxide	0.8	1.7	1.20	0.46	–	–	–	–
9 1H-Pyrrole	0.0	0.6	0.33	0.31	0.7	1.6	1.13	0.45
10 Benzaldehyde	0.0	0.8	0.40	0.40	–	–	–	–
11 Pentadecane	–	–	–	–	0.2	0.6	0.40	0.20
12 5-Methyl-2-furancarboxaldehyde	0.0	0.4	0.23	0.21	0.0	0.3	0.17	0.15
13 Hotrienol	1.4	1.8	1.60	0.20	0.3	0.8	0.57	0.25
14 Phenylacetaldehyde	66.2	68.4	66.53	1.72	–	–	–	–
15 Hexadecane	0.2	0.3	0.27	0.06	0.0	0.3	0.20	0.17

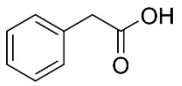
I. Jerković i dr., *Ultrasonics Sonochemistry* 14 (2007) 750-756

Mnogi isparljivi/poluisparljivi spojevi sa kisikom su vodotopljivi
- Ne mogu se izolirati destilacijom!

Table 2 (*continued*)

	HD				USE			
	Min.	Max.	Avg.	δ	Min.	Max.	Avg.	δ
62 Geranic acid*	0.0	1.1	0.53	0.55	0.0	2.2	1.10	1.10
63 3-(2-Hydroxypheyl)-2-propenoic acid	–	–	–	–	0.0	4.0	1.73	2.05
64 2,3-Dihydrobenzofuran (*Coumaran*)	–	–	–	–	0.0	4.9	1.63	2.83
65 Benzoic acid	–	–	–	–	2.5	5.5	4.33	1.61
66 3-Methyl-1H-indole	0.4	0.7	0.53	0.15	–	–	–	–
67 5-Hydrohymethyl-2-furancarboxaldehyde	–	–	–	–	0.0	13.3	6.10	6.72
68 Tetracosane	23.0	27.8	24.93	2.53	0.0	1.8	0.80	0.92
69 Phenylacetic acid	–	–	–	–	20.2	23.5	21.57	1.52
70 Isodihydrocarveol*	–	–	–	–	0.0	1.2	0.57	0.60
71 (*Z*)-9-octedecen-1-ol	–	–	–	–	0.0	2.5	1.0	1.32
72 Hexadecanoic acid (*Palmitic acid*)	0.2	11.0	4.90	5.53	–	–	–	–
Yield (mg/kg)	8.4	12.1	10.17	1.86	21.1	38.6	29.90	8.75

Min., minimal percentage; I_1, retention indices on HP-20M column.
Max., maximal percentage; I_2, retention indices on HP-101 column.
σ, standard deviation; –, not detected on this column.
Avg., average percentage; *, tentatively identified.

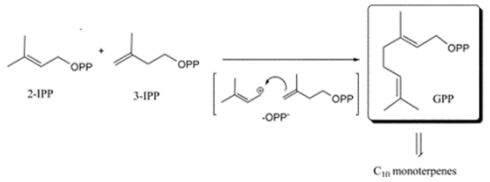


***Robinia pseudoacacia* L.**

I. Jerković i dr., *Ultrasonics Sonochemistry* 14 (2007) 750-756

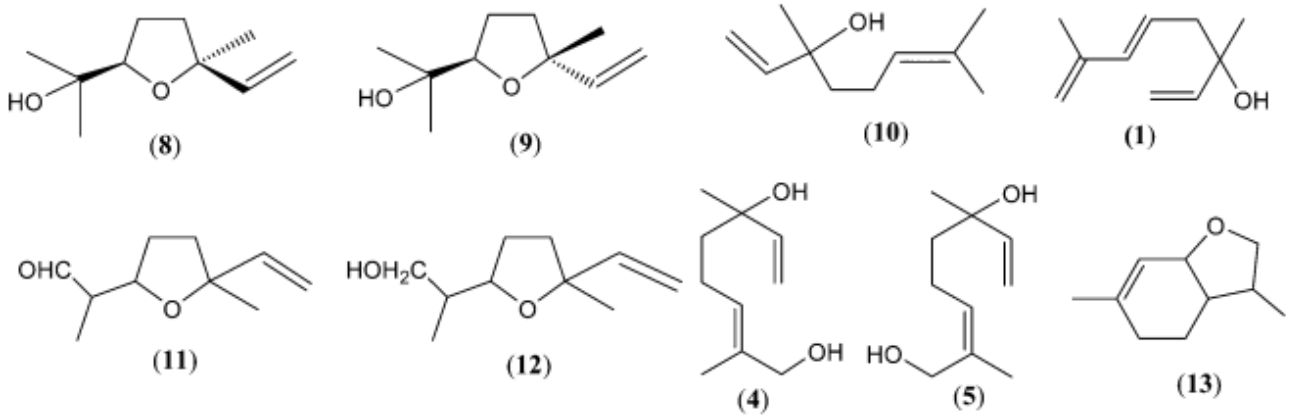
Prikladne metode za izolaciju monoterpena iz meda:

- ultrazvučna ekstrakcija otapalom (USE)
- mikroekstrakcija vršnih para na krutoj fazi (HS-SPME)
- statička ili dinamička ekstrakcija vršnih para (SHS ili DHS)



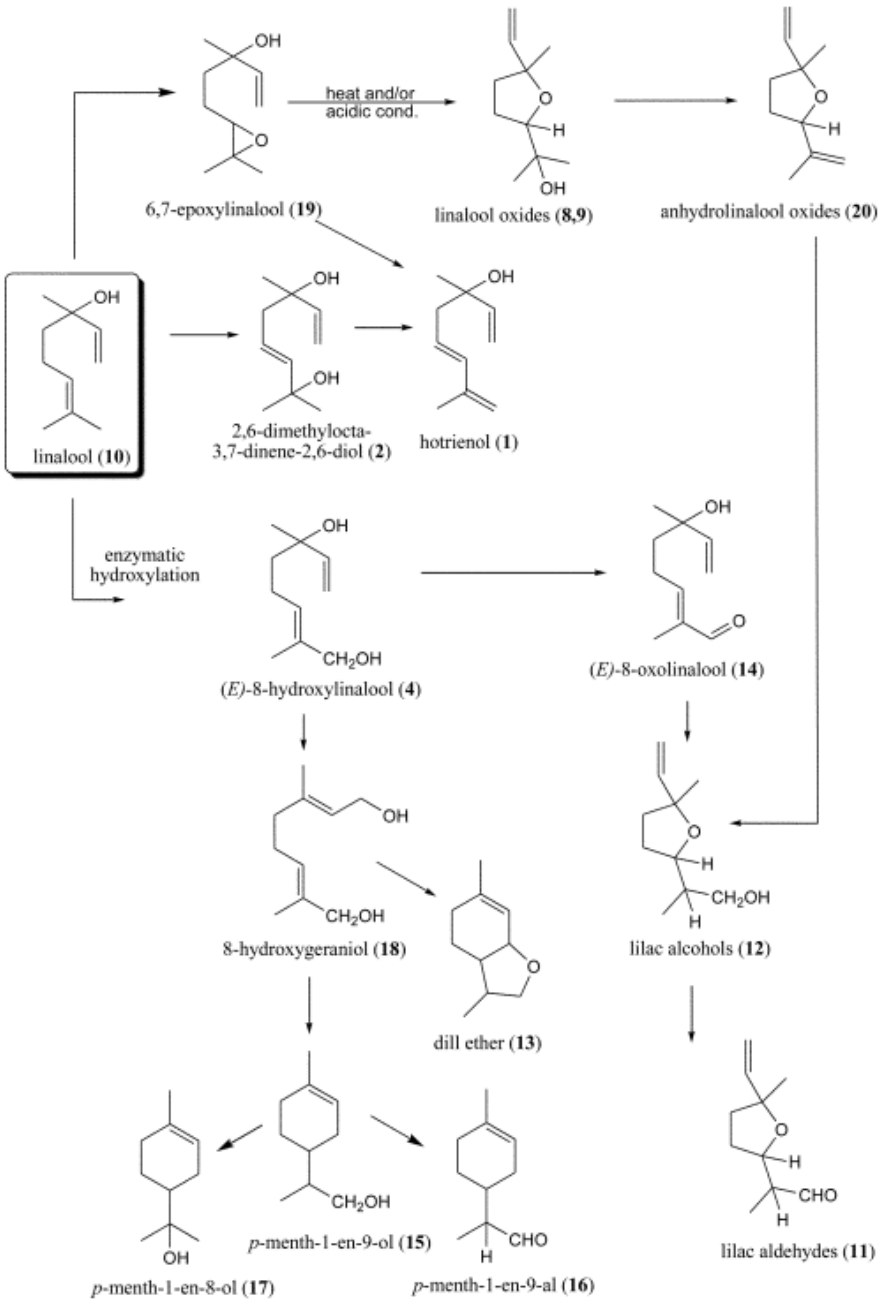
Isparljivi spojevi meda su identificirani kao:

a) spojevi koji potječu iz biljaka (fitokemikalije) u njihovom izvornom obliku
b) produkti pčelinje transformacije izvornih spojeva meda
c) produkti termičkog tretmana i/ili produženog skladištenja



Tipične strukture monoterpena u medu su **derivati linaloola** (10):

- *cis*/*trans*-linalool oksidi (8, 9)
- izomeri aldehida (11)/alkohola (12) jorgovana
- (*E*)/(*Z*)-8-hidroksilinalool (4, 5)
- izomeri etera kopra (13)
- hotrienol



PROBA S LINALOOLOM:

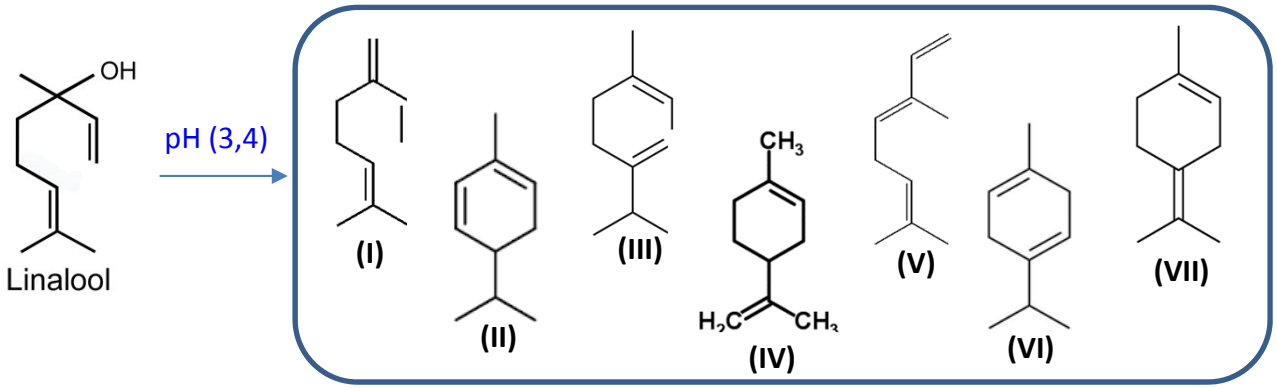
biokonverzija linaloola u zatvorenoj košnici; pčele su hranjene uz dodatak linaloola: nastaju furan/piran linalool oksidi (8, 9) i 2,6-dimetilokta-3,7-dien-2,6-diol (2) - kataliza enzimima pčela; (*E*)-8-hidroksilinalool (4) nastao je hidroksilacijom linaloola (hidroksilaza)

Važni derivati linaloola kao aldehidi jorgovana (11), *cis*/*trans*-anhidrolinalool oksidi (20) i (*Z*)-8-hidroksilinalool (5) nisu dobiveni u probi sugerirajući potrebu biljnih enzima za biokonverziju; slično je i za (*E*)-8-hidroksilinalool (4)

E. Alissandrakis i dr., *Eur. Food Res. Technol.* 231 (2010) 21-25

LINALOOL U KISELIM UVJETIMA:

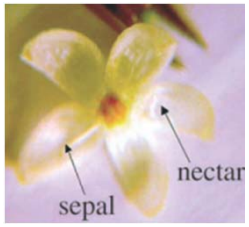
otopina linaloola (1%) u CH_3OH je miješana u zakiseljenoj H_2O (pH 3,4) 10 dana na 30 °C: 3/4 linaloola ostalo je nepromijenjeno, 1/3 pretvorena je u mircen (I), α-felandren (II), α-terpinen (III), limonen (IV), (Z)-β-ocimen (V), (*E*)-β-ocimen (V), γ-terpinen (VI) i terpinolen (VII) → **kiseli uvjeti ne vode do formiranja tipičnih derivata linaloola pronađenih u medu**



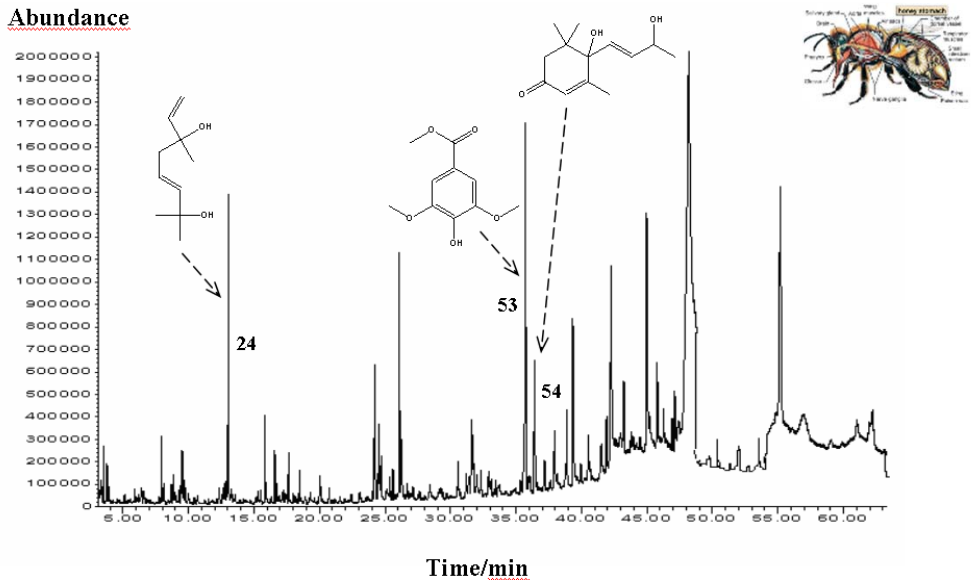
E. Alissandrakis i dr., *Eur. Food Res. Technol*. 231 (2010) 21-25

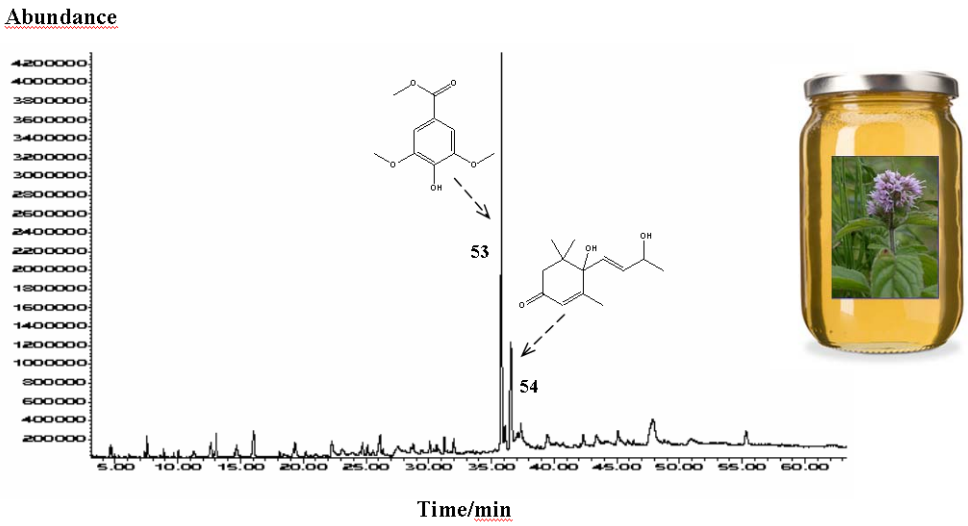
USPOREDBA MEDNI MJEHUR/MED:

u mednom mjehuru sa nektarom lipe (*Tilia cordata* Mill.) pronađeni su monoterpenski alkoholi: 3,7-dimetilokta-1,5-dien-3,7-diol, 3,7-dimetilokta-1,6-dien-3,5-diol i 2,6-dimetilokta-2,7-dienal (vjerojatno nastali djelovanjem glikozidaza na glikozide nektara)



R. Naef i dr., *Chemistry & Biodiversity* 1 (2004) 1870-1879





- ekstrakt mednog mjehura (nakon sakupljanja *Mentha* spp. nektara) sadržavao je metil-siringat, terpendiol I i vomifoliol koji se mogu povezati s biljnim porijeklom; ostali glavni spojevi su bili slični sastavu kutikularnih voskova, a manje feromonima
- metil-siringat i vomofoliol su preneseni u med, dok se terpendiol I djelomično transformirao u hotrienol u zrelom medu (vršne pare)

Jerković et al., *Molecules* 15 (2010) 2911-2924

USPOREDBA CVIJET/MED:

- Usporedba ekstrakata *Citrus* spp. cvjetova i *Citrus* spp. meda pokazala je slične monoterpene; ekstrakti cvjetova 4 *Citrus vrste* (limun, naranča, gorka naranča i tangerina) sadržavali su linalool kao predominanti spoj



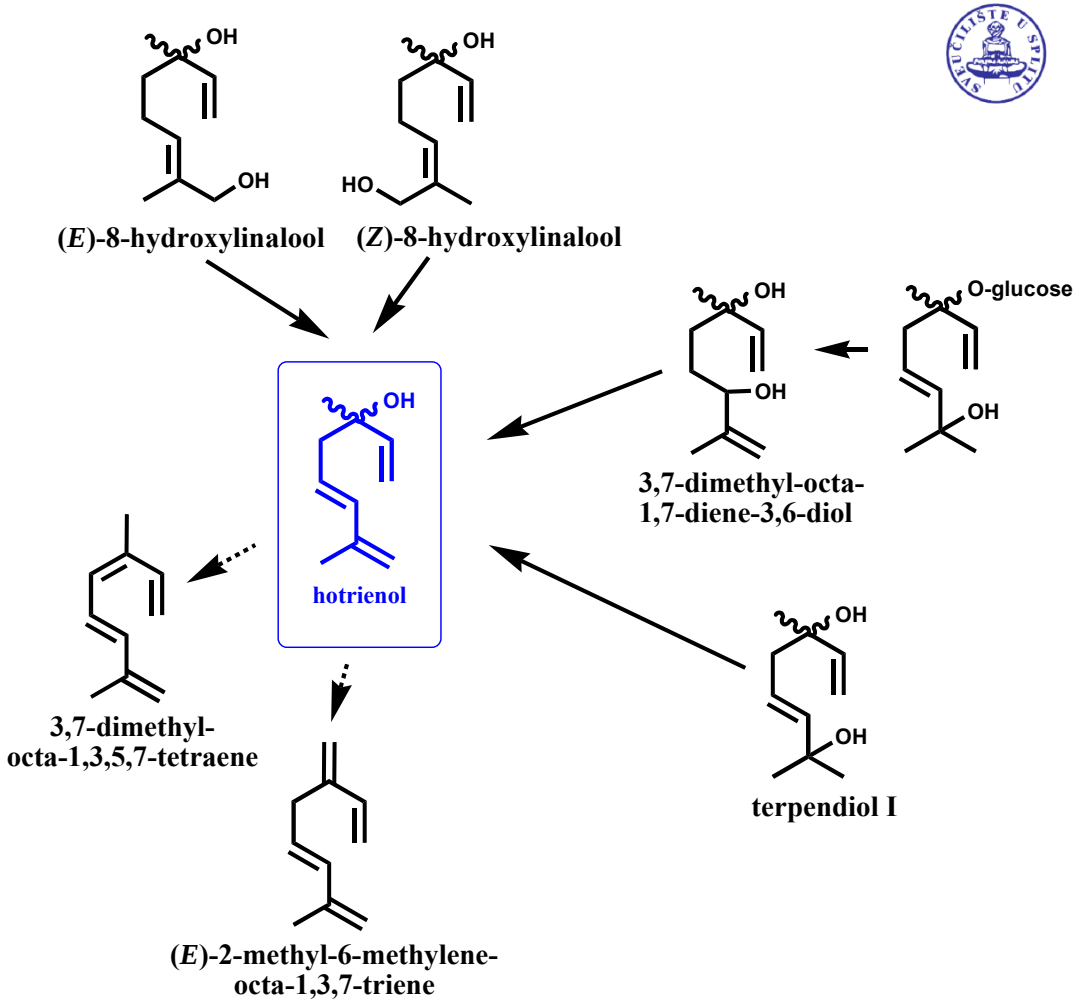
Citrus spp. flowers

- ekstrakti *Citrus* spp. meda: derivati linaloola (više od 80%) i to (*E*)-2,6-dimetilokta-2,7-dien-1,6-diol (dominantan) uz 2,6-dimetilokta-3,7-dien-2,6-diol i (*Z*)-2,6-dimetilokta-2,7-dien-1,6-diol

- osim linaloola, monoterpeni u medu jabuke (uglavnom hotrienol, terpendiol I, (*E*)-8-hidroksilinalool, furan linalool oksidi i car-2-en-4-on) su različiti od onih u cvjetovima jabuke (limonen, car-3-en, α-pinen, α-humulen, terpinolen, geraniol, (*Z*)- i (*E*)-citral i dr.)

Alissandrakis et al., *Food Chem.*, 82 (2003) 575

- **hotrienol** je posebno nestabilan spoj među monoterpenima te je poznati termički artefakt, ali postoje istraživanja koja podržavaju njegovu prirodnu prisutnost u izvornom medu



Eucryphia lucida Baill.

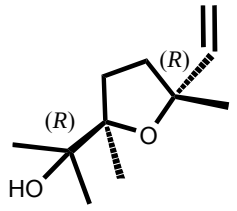
- **hotrienol** je glavi spoj vršnih para meda meda *Eucryphia lucida* Baill., a 2,6-dimetilokta-3,7-dien-2,6-diol i hotrienol su bili glavni spojevi ekstrakata; diol je također identificiran u nektaru

Rowland i dr., *J. Agric. Food Chem.*, 43 (1995) 753

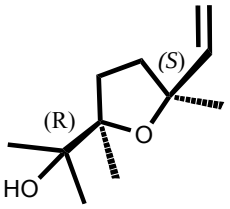
DISTRIBUCIJA KIRALNIH MONOTERPENA U MEDU

- prirodni kiralni terpeni imaju karakterističnu distribuciju enantiomera što ovisi o stereokontroliranim biogenetskim reakcijama

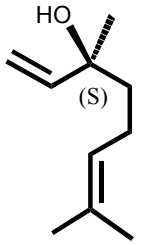
- Podaci o enantiomernom sastavu kiralnih terpena u medu nedostaju
- 2 načina kiralnog odvajanja : 1) 1D GC sa kiralnom stacionarnom fazom, 2) multidimenzijska GC



trans-(2*R*,5*R*)-linalool oxide



cis-(2*S*,5*R*)-linalool oxide



(3*S*)-linalool

- *trans*-(2*R*,5*R*)-linalool oksid, *cis*-(2*S*,5*R*)-linalool oksid i (3*S*)-linalool dominiraju u vršnim parama meda naranče (kiralna GC)

- Monoterpeni meda naranče su izravno korelirani s onima u cvjetovima: (+)-(3*S*)-linalool dominira u vršnim parama cvjetova naranče, a isti enantiomer je pronađen u medu naranče

Pažitná et al., *J. Food Nutr. Res.* 51 (2012) 236.

PRIMJER 1. *Coriandrum sativum* L. med – vršne pare

Coriandrum sativum L.

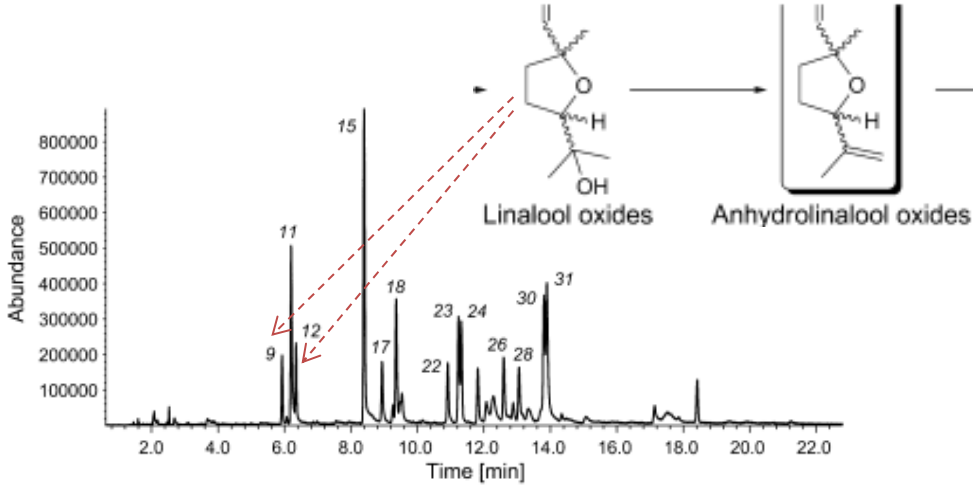
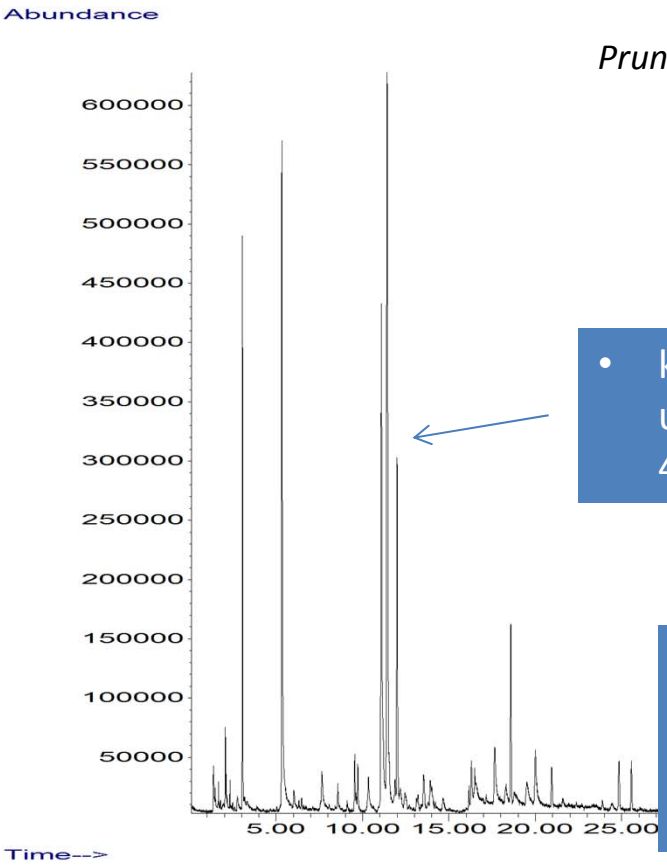


Fig. 1. *Representative chromatogram of coriander honey volatiles obtained by HS-SPME with DVB/CAR/PDMS fiber.* Numbers correspond to those in *Table 2*.

- *trans*-linalool oksidi (11.1%; 14.6%) dominirali su vršnim parama uz *cis*/*trans*-anhidrolinalool okside (5.0%; 5.9%), izomere aldehida/alkohola jorgovana (14.9%; 13.8%) i *p*-ment-1-en-9-al (15.6%; 18.5%)
- dva izomerna anhidrolinalool oksida su identificirana prvi put u medu *Citrus* spp.
- (*E*)/(*Z*)-2,6-dimetilokta-2,7-dien-1,6-diol (17.8%; 16.1%)) je pronađen u USE ekstraktima, ali ne u vršnim parama

Jerković et al., *Chem. Biodivers.* 10 (2013) 1549

PRIMJER 2. *Prunus cerasus* L. med – vršne pare



Prunus cerasus L.

- kromatografski profil vršnih para karakteriziran je uglavnom izomerima aldehida jorgovana (ukupno 48.3%)

- aldehidi jorgovana posjeduju miris koji se opisuje kao “ugodan, sladak, svjež, cvjetni”; budući je njihovo granica detekcije mirisa niska njihov utjecaj na aromu meda može biti značajan

Kuš, Jerković et al., *Nat. Prod. Commun.* 8 (2013) 651.

USPOREDBA SASTAVA ISPARLJIVIH SPOJEVA MED/ETERIČNO ULJE

Plant source	Major honey terpenes	Method of isolation	Major essential oil terpenes	Method of isolation
***L. stoecheas* L.** **(French lavender)** 	• -	• **CH_2Cl_2/ SDE**	• **flowers**: camphor, 1,4-cineol, γ-terpinene, 1,8-cineol, linalool, linalyl acetate	• **HD**
***L. angustifolia x latifolia* L.** **(lavandin)** 	• *cis*-/*trans*-linalool oxide, linalool, hotrienol, epoxylinalool isomers, myrtenol, 2,6-dimethylocta-3,7-dien-2,6-diol, 1,8-cineol isomers, 1-hydroxylinalool, farnesol • -	• **SPE** • **CH_2Cl_2/SDE**	• **flowers:** linalool, linalyl acetate, α-terpineol, lavandulol, 1,8-cineol, *cis*-/*trans*-linalool oxide	• **HD** • **SD**
***L. latifolia* L.** **(Spike lavender)** 	• *cis*-/*trans*-linalool oxide, linalool, hotrienol, α-terpineol, epoxylinalool isomers, myrtenol, *p*-cymen-8-ol, 2-hydroxycineole, 2,6-dimethylocta-3,7-dien-2,6-diol, 1,8-cineol isomers, 1-hydroxylinalool	• **SPE**	• **flowers:** linalool, camphor, 1,8-cineol, borneol, α-terpineol, terpinen-4-ol, farnesol	• **HD**

Plant source	Major honey terpenes	Method of isolation	Major essential oil terpenes	Method of isolation
***L. angustifolia* L. (English lavender)** 	• -	• **CH_2Cl_2 /SDE**	• **flowers:** 1,8-cineol, borneol, camphor	• **HD**
***L. hybrida* Reverchon II (lavandin)** 	• hotrienol • hotrienol, 3,7-dimethylocta-1,5-diene-3,7-diol	• **HS-SPME** • **USE**	• **flowers:** linalool, 1,8-cineole, limonene, *cis*-β-ocimene, camphor, linalyl acetate, terpinen-4-ol, alloocimene, α-terpineol	• **HD**
***Coriandrum sativum* L.** 	• *cis*-/*trans*-linalool, *cis*-/*trans*-anhydrolinalool oxide, lilac aldehyde/alcohol isomers, *p*-menth-1-en-9-al • (*E*)/(*Z*)-2,6-dimethylocta-2,7-diene-1,6-diol	• **HS-SPME** • **USE**	• **flowers:** linalool; seeds: linalool, geranyl acetate, γ-terpinene, linalyl acetate, others	• **HD**

Plant source	Major honey terpenes	Method of isolation	Major essential oil terpenes	Method of isolation
***Mentha* spp.** ***(Mentha aquatica* L. *and Mentha pulegium* L.)** 	• hotrienol, *cis*-/*trans*-linalool oxides, linalool, neroloxide • 3,7-dimethylocta-1,5-dien-3,7-diol	• **HS-SPME** • **USE**	• ***Mentha aquatica* L. (leaves and flowers):** menthofuran, 1,8-cineole, *trans*-caryophyllene, γ-cadinene, viridiflorol, linalool, others • ***Mentha pulegium* L. (leaves and flowers)**: menthone, pulegone, neo-menthol	• **HD** • **HD**
***Rosmarinus officinalis* L.** 	• lilac aldehyde isomers • *cis*-/*trans*-linalool oxides, hotrienol, car-2-en-4-one, nerol, geraniol • hotrienol, car-2-en-4-one, 2,6-dimethyl-octa-3,7-diene-2,6-diol • car-2-en-4-one, 2,6-dimethyl-octa-3,7-diene-2,6-diol, hotrienol, *cis*-/*trans*-linalool oxides	• **SDE** • **SDE** • **SE** • **SPE**	• **leaves and flowers:** α-pinene, 1,8-cineole, verbenone, camphor, borneol, bornyl acetate, *trans*-caryophyllene, camphene, linalool	• **HD**

ZAKLJUČCI

- terpeni do C_{15} su pronađeni kao glavne komponente eteričnih ulja, ali med od iste biljne vrste pokazuje samo djelomičnu sličnost sa stavom ulja

- linalool derivati kao što su *cis*- i *trans*-linalool oksidi, hotrienol, izomeri aldehida ili alkohola jorgovana, (*E*)/(*Z*)-8-hidroksilinalool i sl. su najzastupljeniji u različitim vrstama meda

- samo nekoliko specifičnih monoterpena je pronađeno kao kemijski markeri u medu (npr. izomeri anhidrolinalool oksida)

- hotrienol je osobito nestabilan spoj poznat kao termički artefakt, ali moguće je da se identificira u izvornom medu (bez zagrijavanja)

- različite metode ekstrakcije isparljivih spojeva meda (npr. USE ili HS-SPME) bez zagrijavanja uzorka se trebaju kombinirati radi dobivanja potpunog profila manje i više isparljivih spojeva u medu

HVALA NA PAŽNJI!

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