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Ragweed components in honey

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SUMMARY

The aim of this research was to prove that the syrup containing ragweed used for feeding the bees was transferred to the honey. To reach this goal we developed a method to analyse the volatile components of the samples. We applied two sample preparation methods. The first one was SPME (Solid Phase Micro Extraction) preparation from the headspace of all of the samples. In the second case only the raw leaves of the ragweed were extracted with n-hexane and dichloromethane to complete the SPME results. The measurements were performed on GC-MS equipment. Chromatograms and data evolution showed that the components of the ragweed were also present in honey so they were transferred by bees.

Keywords: *ragweed, honey, honey product, honey keeper, SPME extraction, volatile components, gas-chromatography, mass spectrometry*

ÖSSZEFOGLALÁS

A kísérlet célja bebizonyítani, hogy a méhek etetésére felhasznált parlagfű tartalmú szirup a méhek közvetítésével átkerül a késztermékbe. Módszerfejlesztésre került sor, mely a minták illó összetevőinek elemzésére irányul. Két mintaelőkészítési módszert alkalmaztunk. Az egyikben SPME (szilárd fázisú mikroextrakció) módszerrel a minták gőzterét elemeztük. A másik esetben oldószeres extrakciót végeztünk n-hexánnal és diklórmetánnal az SPME módszer kiegészítéseképpen. A mérések GC-MS módszerrel történtek. A kromatogramok és az adatkiértékelés alapján kijelenthető, hogy a parlagfű bizonyos komponensei a méhek közvetítésével a kész méhtermékben is megjelennek.

Kulcsszavak: *parlagfű, méz, méztermék, méhészet, SPME extrakció, illó komponensek, gázkromatográfia, tömegspektrometria*

INTRODUCTION

If people hear the word ragweed, they think immediately to allergy. Common ragweed has a very serious health risk for humans in terms of pollen allergy. Pollen of ragweed is the most potent causing of hay fever, allergic rhinitis or severe asthma-like symptoms (Buttenschøn et al. 2010).

Examination of the allergens in ragweed pollens started several decades ago (Dankner et al. 1951, Frankel et al. 1955, Sehon et al. 1956). New studies investigated the concrete allergenic components (Moingeon et al. 2014, Wopfner et al. 2009). But only few studies are available where authors examined the chemical composition (Chubinidze and Molodinashvili 1983, Chubinidze et al. 1984, Chalchat et al. 2004).

Even though that ragweed is an invasive species and its pollen is very allergic its beneficial effects are also known. In some studies, it is used as allergen immunotherapy (Creticos et al. 1996) and there are other known but not scientific treatments.

There are only few papers available in connection to ragweed extracts and its effects (Roedel and Thornton 1942, Tóth et al. 2011, 2012).

Honey is a well-known alternative medicine as well (Szalay and Halmágyi 1998, Molan 1999). Honey keepers got the idea to feed the bees with different medicinal plant extracts to make them stronger and healthier. One of the used plants was common ragweed *(Ambrosia artemisiifolia)* (Patent, P 15 00061 Daróczi 2014).

After that they recognised that the honey made by bees got these extract of herbals has similar taste and colour to the plant which was made from; they decided to produce it for human consumption (Sáfián 2012). In this way they combined the beneficial effects for health of both honey and ragweed, but since bees got extra nutrition the product is not allowed to trade as honey but is labelled as "MORETHENHONEY" (in Hungarian: „TÖBBMINTMÉZ").

Some volunteers who are strong allergic to ragweed tested this product instead of the allergic medicine. Most of them gave the feedback that their allergic symptoms get reduced and they do not need to take the allergic pills in (Daróczi 2014).

The aim of our research is to prove that the volatile components of the ragweed are also present in the syrup and honey samples. To convince this assumption we developed a SPME and an extraction sample preparation followed by GC-MS (Gas Chromatography – Mass Spectrometry) method (Cuevas-Glory et al. 2007). Based on the results the similarity between the different samples is obvious so the volatile components of the plant were present in the syrup samples and some of them were transferred to the bee product by the bees.

MATERIALS AND METHODS

Samples

The available samples were the following:

- raw ragweed leaves which were used to prepare the syrup (one mixed sample, leaves from more plant),
- the syrup which was used to feeding the bees (four differently prepared syrup),
- the bee product (two bee product, made from the syrup transferred by bees).

Sample preparation

The SPME sample preparation was made with manual tool. The honey, the syrupy and the raw leaf samples were placed into lockup sample vials equipped with septa. The extraction was made through the septum, from the headspace.

- SPME fibre: 85 µm poliacrylate
- Extraction time: 1 hour
- Extraction temp.: 50 °C
- Desorption time: 30 sec
- Desorption temp.: 200 °C (in the GC injection port)

The other method was the extraction with n-hexane and dichloromethane. In this case the raw plant leaves were rubbed in a mortar with a little quartz sand and with 20–20 millilitres of the two solvent above. The sampling was made from the extractions of the leaves with manual syringe. The injected amount was 1 µl.

Analysis

The GC-MS measurements were executed on a 5890 Series II – 5971 mass spectrometer system. The equipment has split-splitless injector.

Gas-chromatograph parameters:

- column: HP-5 capillary column×0.25 mm×0.25µm,
- carrier gas: helium (1 ml/min, constant pressure),
- temperature program: 40 °C 2 min followed by 5 °C/min up to 200 °C,
- analysis time: 44 min,
- injector temperature: 200 °C,
- injector liner: liner without packing.

Mass spectrometric parameters:

- transfer line temperature: 280 °C,
- ionization: 70 eV (for library searchable spectra),
- mass range: 10–500 AMU.

Hewlett-Packard GC-MS Chemstation rev.3 was used for system control, data acquisition, and data evaluation. The components were identified with Wiley and NIST databases and retention indices from different manuals (Cserháti 2010, Tarján and Takács 2012).

RESULTS

Table 1 shows only the components identified with high certainty. We calculated the retention indices of the components using the known retention indices of identified straight-chain saturated fatty acid ethyl esters from the TIC (Total Ion Chromatogram). The identification was confirmed by the comparison of the data from the handbooks of indices data (Adams 2009). In this way we were able to identify almost sixty components in the samples. The raw ragweed and the syrups contains more than hundred components *(Figure 1)* but some of them is present in very low concentration so the determination was uncertain.

One of the most common and in high amount occurring component of ragweed is Germacrene D which was determined in earlier studies (Chalchat et al. 2004). It is easy to see on *Figure 1*, that Garmacrene D is one of the predominant components it has the highest peak on the chromatogram. This molecule is a volatile sesquiterpen which has very important role in antimicrobial and insecticide activity of the plant (Kiran and Devi 2007).

Figure 2 shows much less aroma compound with much smaller peak area values than *Figure 1*. Most of these components are also present in the raw ragweed as well, but in higher concentration. The bee product samples have about forty to fifty components but we were able to identify less than twenty from these. Germacrene D was not found in measurable quantity. But there were some components determined which were not present in the raw plant and syrup samples.

CONCLUSION

Our objective was to prove that the ragweed containing syrup was taken to the hive by bees. To confirm this hypothesis an SPME sample preparation method (followed byGC-MS analysis) was developed. The presented results of the measurements show, that some of the components of the ragweed containing syrups were transferred to the honey by the bees. Some of the volatile components of the ragweed are present in the prepared bee product. The honey contains other volatile molecules, which contains the honey made from the natural nectar sources. It proves indirectly that other, even non-volatile components of the plant can be transferred to the honey therefore the active ingredients can become the components of honey.

Investigation of the allergic and medicinal effect was not included in the goal of this project.

Figure 1: **TIC of raw ragweed**

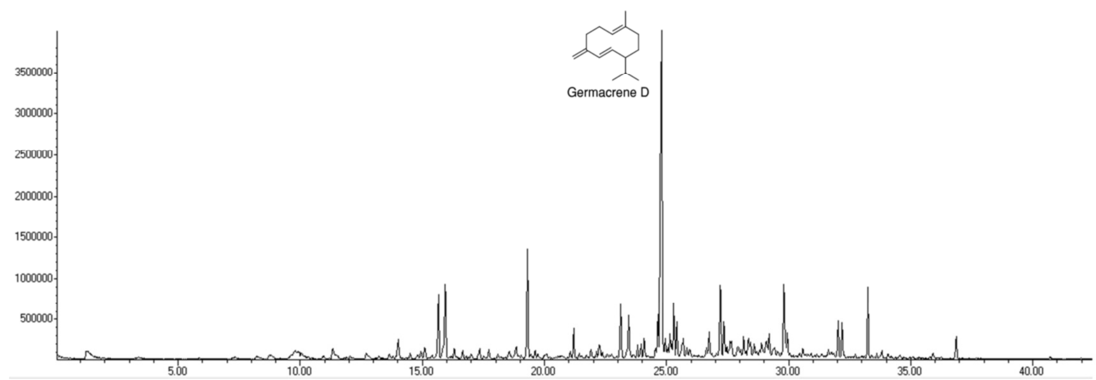


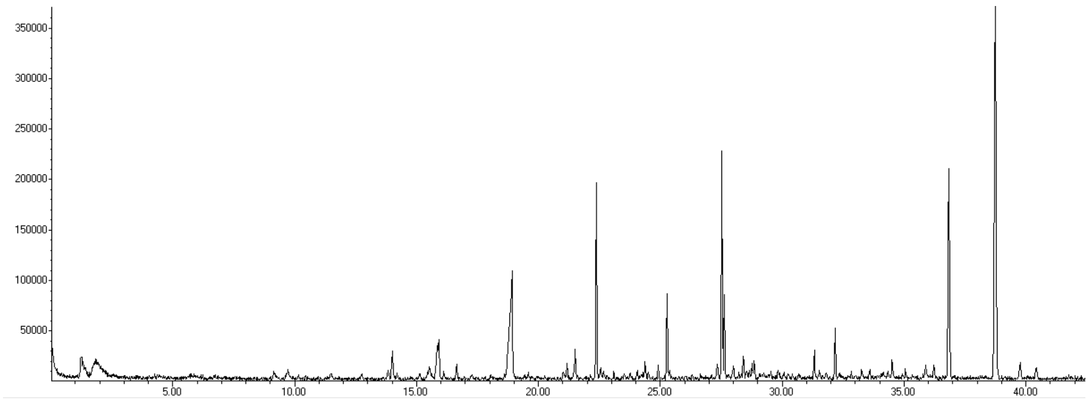
Table 1.

The identified components

Nr	RI[1]	Component	CAS[2]	R[3]	V[4]	VS[5]	S[6]	SF[7]	H1[8]	H2[9]	S1[a]	S2[b]
1	932	alpha-Pinene	000080-56-8	X							X	X
2	988	beta-Myrcene	000123-35-3								X	X
3	967	Hexanoic acid	000142-62-1	X	X	X		X	X	X		
4	1024	Limonene	000138-86-3	X							X	X
5	1095	Linalool	000078-70-6	X	X	X	X				X	X
6	1101	Benzyl Alcohol	000100-51-6	X	X	X	X	X				
7	1106	Phenylethyl Alcohol	000060-12-8	X	X	X	X	X	X	X		
8		Benzoic acid	000065-85-0					X	X	X		
9	1158	trans-3-Pinanone	015358-88-0	X	X		X					
10		4-ethyl-Phenol	000123-07-9		X	X	X	X				
11	1165	Borneol	000507-70-0	X	X	X	X	X				
12	1167	Caprilic acid	000124-07-2		X	X		X	X	X		
13		Creosol	000093-51-6		X	X						
14		1,4-dimethoxy- Benzene	000150-78-7		X			X				
15	1186	Terpineol	010482-56-1			X	X					
16	1196	Ethyl caplrylate	000106-32-1		X			X				
17	1204	Verbenone D	000080-57-9	X	X							
18		2,3-dihydro-Bezofuran	000496-16-2			X	X	X				
19	1226	Carveol	001197-07-5	X	X							
20	1267	Nonanoic acid	000112-05-0	X	X	X	X	X	X	X		
21	1284	Bornyl acetate	000076-49-3	X	X	X	X	X			X	X
22	1289	Thymol	000089-83-8	X	X	X	X	X				
23		2-methyl-Naphthalene	000091-57-6	X	X							
24		Ethyl pelargonate	000123-29-5		X	X		X				
25		Ethyl-3-phenylpropionate	002021-28-5		X			X				
26	1356	Eugenol	000097-53-0	X	X	X	X	X	X			
27		Cocos aldehyde	000104-61-0	X	X	X						
28	1364	Capric acid	000334-48-5		X	X	X	X	X	X		
29	1395	Decanoic acid ethyl ester	000110-38-3		X			X	X	X		
30	1408	Caryophyllene	000118-65-0	X	X	X			X		X	
31	1417	beta-Caryophyllene	000087-44-5	X	X	X	X	X			X	
32	1440	(Z)-beta-Farnesene	026560-14-5	X	X							
33	1454	(E)-beta-Farnesene	028973-97-9	X							X	X
34	1452	alpha-Humulene	006753-98-6	X	X			X				
35	1478	gamma-Muurolene	030021-74-0				X	X				
36	1484	Germacrene D	023986-74-5		X	X	X	X			X	X
38	1505	alpha-Farnesene	000502-61-4	X					X	X	X	X
39	1505	beta-Bisabolene	000495-61-4	X		X					X	
40	1522	delta-Cadinene	000483-76-1	X	X			X				
41	1565	Dodecanoic acid	000143-07-7		X	X	X	X				
42	1577	(-)-Spathulenol	006750-60-3	X		X	X	X				
43	1582	Caryophyllene oxide	001139-30-6	X		X	X	X				
44	1592	Viridiflorol	006750-60-3	X	X		X	X			X	
45	1594	Dodecanoic acid ethyl ester	000106-33-2		X	X	X	X	X	X		
46	1602	Ledol	000577-27-5		X			X	X	X	X	
47		Laevojunenol	030951-17-8		X	X	X	X				
48	1626	Benzophenone	000119-61-9						X	X		
49	1652	alpha-Cadinol	000481-34-5		X	X		X				
50	1675	Cadalene	000483-78-3		X	X	X					
51		Tetradecanoic acid	000544-63-8			X		X				
52	1795	Tetradecanoic acid ethyl ester	000124-06-1		X	X	X	X	X	X		
53		Hexahydrofarnesyl acetone	000502-69-2	X	X	X	X	X				
54		Pentadecanoic acid ethyl ester	001114-00-5			X		X				
55	1959	Palmitic acid	000057-10-3	X	X	X	X	X				
56		Ethyl -9-hexadecenoate	054546-22-4		X	X		X				
57	1992	Hexadecanoic acid ethyl ester	000628-97-7	X	X	X	X	X	X	X		
58	2196	Linoleic acid ethyl ester	0000544-3-4	X	X	X	X	X	X	X		
59		Ethyl Oleate	000111-62-6		X	X	X	X				

Note: [1]Retention Index (Adams 2009), [2]Chemical Abstracts Service, [3]Raw ragweed leaves, [4]Ragweed vinegar, [5]Syrup with ragweed vinegar, [6]Syrup, [7]Syrup test feeding, [8]Bee product 1, [9]Bee product 2, [a]Extraction with n-hexane, [b]Extraction with dichloromethane

Figure 2: **TIC of the bee product**



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