Acta Alimentaria, Vol. 32 (4), pp. 395–403 (2003)

Preliminary communication

A COMPARATIVE ANALYSIS OF HUNGARIAN ROBINIA AND MILKWEED HONEYS BASED ON THEIR CHEMICAL AND PHYSICAL CHARACTERISTICS

ZS. KASPER-SZÉL[a]*, M. AMTMANN[b], A. TAKÁTS[a] and Á. KARDOS-NEUMANN[c]

[a] Institute for Small Animal Research, 2100 Gödöllő, P.O. Box 417. Hungary
[b] Department of Food Chemistry and Nutrition Science, Szent István University, H-1118 Budapest, Somlói u. 14–16. Hungary
[c] Central Food Research Institute, H-1022 Budapest, Herman Ottó u. 15. Hungary

(Received: 15 July 2002; accepted: 10 July 2003)

Sugar composition, pH, invertase and diastase activity and colour of 7 robinia (*Robinia pseudoacacia*) and 8 milkweed (*Asclepias syriaca*) honey samples were compared, all purchased from producers and wholesalers. Milkweed honeys proved to be of darker colour and more acidic. The two unifloral honeys showed statistically significant differences between their diastase and invertase activities, milkweed honey showed higher enzyme activity values than robinia honey. The fructose to glucose ratio was 1.58 in case of robinia honey and 1.28 in case of milkweed honey. There was also a significant difference between the di- and trisaccharide content of the two unifloral honeys.

Keywords: robinia honey, milkweed honey, sugar content, diastase, invertase

Robinia is the most important melliferous plant of Hungary. It provides not only one of the largest quantities of honey, but it stands on the first place also because of its quality, unique flavour characteristics and because it sells well in the world market.

Robinia honey is transparent, its colour ranges from almost colourless to light yellow. It does not or hardly crystallizes due to the presence of a relatively large quantity of fructose, and it has a characteristic aroma. A honey to be classified as robinia honey has to contain at least 10% robinia pollen. Robinia honey is collected by bees at the end of May and extracted in June.

Milkweed is an industrial plant originating from North America which grows naturally in huge quantities in Hungary from the 1980s in certain areas (Bács-Kiskun, Jász-Nagykun-Szolnok, Somogy and Pest Counties). Because of its good melliferic features milkweed has become significant from apiarian point of view. Milkweed honey is very similar to robinia honey, as for its appearance, colour and composition. It is impossible to identify this honey based on its pollen content, because it does not contain pollen due to the floral structure of the plant. Milkweed honey is extracted in June or July.

* To whom correspondence should be addressed.
Fax: +36-28-430-184; e-mail: szel@katki.hu

0139-3006/2003/$ 20.00

GULYÁS (1993) described milkweed nectar and honey analysis in details referring to the similarity of robinia and milkweed honey.

FÖLDHÁZI (1994) examined the sugar composition of honeys of different botanical origin including robinia and milkweed honey. According to his results, the two unifloral honeys were very similar in their sugar content but there was a difference in their fructose-glucose ratio: F/G was 1.7 (n=7) in robinia and 1.4 (n=3) in milkweed honey.

SZABÓ (2001) compared the sugar composition of 10 Hungarian unifloral and several mixed honey types. According to her results, robinia honey has a higher fructose-glucose ratio than milkweed honey (1.64; n=5 and 1.36; n=3, respectively). Robinia honey contains more turanose and isomaltose than milkweed honey, but in milkweed honey more maltose, melezitose and erlose have been found.

MATEO and BOSCH-REIG (1997) determined the levels of fructose, glucose, maltose, maltulose, kojibiose, isomaltose, raffinose, erlose and melezitose in 7 different Spanish unifloral honey types (rosemary, orange blossom, lavender, etc.). There were significant differences among the honey types in relation to sugar composition. Fructose, glucose, sucrose, maltose and glucose/water ratio were selected by discriminant analysis as the better parameters for the correct classification of the honey samples into their parent types.

KRAUZE and ZALEWSKI (1991) determined 18 chemical and physical parameters of nectar and honeydew honeys and analysed the results statistically by the method of principal component analysis. On the plots of principal component loadings, the honeys were divided into the following groups: robinia-, rape-, linden-, floral-, heather- and honeydew honey. Among examined honey types robinia contained the most fructose (44.15%) and sucrose (2.31%) and the least glucose (28.44%). Robinia honey compared with other honey types was characterized by low pH value (3.88) and diastase number (11.5).

The two unifloral honeys (robinia and milkweed) seem to be very similar not only in their appearance but also in their chemical composition. The flowering period of robinia and milkweed may overlap and their honeys might mix. The question arises whether robinia honey mixed with milkweed has a lower quality compared to the homogeneous one. To answer this question the chemical composition of the two unifloral honeys should be known.

In our earlier studies the activity of diastase and glucose-oxydase enzymes was compared in robinia and milkweed honey (SZÉL et al., 2002). These results showed that both diastase and glucose-oxydase activities of milkweed honey were significantly higher than those of robinia honey.

PERSANO and co-workers (1990) determined the diastatic activity in 12 groups of unifloral honey by Schade-White-Hadorn method. They examined 76 samples of robinia honey where the mean DN number was 8.4 (± 2.9).

In this study, 7 robinia honeys and 8 milkweed honeys were compared regarding their chemical composition, pH, sugar content in dry matter, invertase and diastase activity. Our study was extended with colour and pollen analysis, too.

1. Materials and methods

Seven robinia and 8 milkweed honey samples from different areas of the country were analysed with two replicates of each. All were harvested in 2001 and purchased from producers, Aranynektár Ltd and Mézvilág Ltd.

Pollen analysis was carried out by the Lukács Quality Control and Services Ltd. The robinia honey samples all contained at least 12% robinia pollen.

Colorimetry was performed by a Lovibond colorimeter, colours compared to a No. 4/55 colour disc, and according to it honeys can be categorized based on Pfund scale (KISS, 1983):

Colour range	Colour value mm on Pfund scale
Water white	0–8 mm
Extra white	9–17 mm
White	18–34 mm
Extra light amber	35–51 mm
Light amber	52–85 mm
Dark amber	86–115 mm and above 115 mm

pH was measured by a Radelkis pH meter according to the method described in HUNGARIAN STANDARD (1980a).

Diastase activity was measured according to the Schade-White-Hadorn method (HUNGARIAN STANDARD, 1981). The Schade method uses starch as a substrate and determines the diastase activity expressed in diastase number (DN number). One diastase unit shows that how much of a 1% starch solution can be degraded in one hour at 40 °C by the diastase enzyme content in 1 g of honey. The invertase activity was measured according to Siegenthaler's method (SIEGENTHALER, 1977). In this method *p*-nitrophenyl-α-D-glucopyranoside (pNPG) (Fluka, Buchs, Switzerland) was used as an enzyme substrate. The IN (invertase number) indicates the amount of sucrose per g hydrolysed in one hour by the enzymes contained in 100 g of honey under test conditions.

Sugar composition was analysed by HPLC according to Szabó's method presented in her MSc thesis (SZABÓ, 2001). HPLC was performed on a Jasco instrument containing Jasco PU-980 HPLC pump (Jasco, Tokyo, Japan), Rheodyne Model 7725 injector with a 20:l loop and an ERC-7515 A RI detector (ERC.INC., Saitama Pref, Japan). The liquid chromatographic column was Supelcosil LC-NH_2, 5 μm, 250×4.6 mm (Supelco, Bellefonte, USA). The mobil phase was acetonitril-water (75:25, v/v) at a flow rate of 1 ml min^{-1}. A Jasco-Borwin 1.5 software was used to record chromatograms and integrate peak area of sugars. In case of 8 sugars an external calibration was employed. Except for erlose and melezitose all sugar standards were adequately separated. The retention times are following: fructose: 5.97 min; glucose: 6.48 min; saccharose: 8.17 min; turanose: 8.66 min; maltose: 9.23 min; isomaltose: 10.38 min; erlose + melezitose: 11.80 min.

Acetonitril and water were HPLC grade (Reanal, Budapest, Hungary). The sugar standards except turanose and erlose were obtained from Supelco (Bellefonte, USA). Turanose originated from Fluka (Buchs, Switzerland) and erlose was obtained from Sigma (Steinheim, Germany).

Dry matter was determined by a Zeiss-Abbe refractometer as it is described in the HUNGARIAN STANDARD (1980b).

2. Results

According to the colorimetry results, all robinia honey samples fell into the 0–8 mm range of Pfund colour scale, the so-called "water white" category. One of the milkweed honey samples belonged to the water white category, 7 samples belonged to the darker shade of "extra white" colour.

The pH values, the diastase and invertase activity values of the honey samples are presented in Table 1. All results were statistically processed with the Student's *t*-test.

The mean pH value of robinia honeys was 3.90, that of milkweed honeys was 3.63, so milkweed honeys proved to be slightly more acidic. The difference between the pH values of the two unifloral honeys was statistically significant (P=0.004). The pH value of robinia honeys was very close to the results of KRAUZE and ZALEWSKI (1991) (3.88, 3.83–3.98).

The mean diastase number of milkweed honeys was 23.58, their invertase number was 13.56; in case of robinia honeys the mean diastase number was 12.93, invertase number was 6.04. The activity of both enzymes is significantly higher in milkweed honey. The diastase values of the two unifloral honeys are similar to our results from an earlier study, when the mean diastase number was 24.48 (n=8) in milkweed honeys and 16.28 (n=10) in robinia honeys (SZÉL et al., 2002). Although PERSANO and co-workers (1990) measured lower DN number (8.4, 3.1–15.0) in robinia honey than that of ours, KRAUZE and ZALEWSKI (1991) had similar value (11.5, 8.3–13.9) to ours.

The standard deviation of both enzyme activity and pH values were higher in the case of milkweed honeys, which can be explained by the fact that robinia honey samples are more homogeneous because of the larger amount produced.

The percentages of the sugar content in dry matter are presented in Table 2. The robinia honeys contained 50.26% fructose and 32.00% glucose on average; the milkweed honeys contained an average of 46.63% fructose and 36.33% glucose. The fructose and glucose content of the two unifloral honeys is significantly different. The fructose-glucose ratio of robinia honeys was an average of 1.58, and that of milkweed honeys was 1.28. One robinia and one milkweed honey chromatogram are shown in Fig. 1 and Fig. 2. KRAUZE and ZALEWSKI (1991) measured lower fructose (44.5, 40.67–48.12) and glucose (28.44, 25.80–30.80) and higher sucrose (2.31, 1.05–3.56) content than we did.

Table 1. Diastase-, invertase numbers and pH value in two honey types

Robinia honey				Milkweed honey			
Sample No.	Diastase number	Invertase number	pH value	Sample No.	Diastase number	Invertase number	pH value
1	10.59	8.57	3.81	1	34.15	16.21	3.61
2	12.48	2.54	3.80	2	13.33	1.22	3.70
3	10.90	10.28	4.00	3	19.05	21.27	3.96
4	10.90	5.07	4.03	4	20.00	15.08	3.53
5	15.00	5.08	3.87	5	26.68	14.49	3.80
6	14.66	4.87	3.90	6	27.62	13.93	3.68
7	16.00	5.90	3.87	7	19.40	10.95	3.36
8				8	28.40	15.39	3.43
min.	10.59	2.54	3.80	min	13.33	1.22	3.36
max.	16.00	10.28	4.03	max	34.15	21.27	3.96
$\bar{x}$	12.93	6.04	3.90	$\bar{x}$	23.58	13.56	3.63
s	±2.26	±2.58	±0.09	s	±6.73	±5.76	±0.19
P value	<0.05	<0.05	<0.05				

min-max: range of measured values
$\bar{x}$: mean value
s: standard deviation
P value: Student's *t*-test

Table 2. Sugar contents in two honey types

Parameters	Robinia honey (n=7)					Milkweed honey (n=8)			
	$\bar{x}$	min.	max.	s	P	$\bar{x}$	min.	max.	s
Fructose % a	50.26	46.55	53.25	±2.12	<0.001	46.63	45.08	48.74	±1.37
Glucose % a	32.00	26.92	34.72	±2.74	<0.05	36.33	34.80	37.20	±0.81
Saccharose % a	0.79	0.06	1.75	±0.64		0.60	0.08	2.70	±0.87
Turanose % a	4.25	3.75	5.46	±0.59	<0.001	2.98	2.28	4.12	±0.57
Maltose % a	2.16	1.21	2.95	±0.65	<0.05	3.20	1.00	4.43	±1.07
Isomaltose % a	0.60	0.40	0.87	±0.16		0.48	0.27	0.81	±0.20
Erlose + melezitose % a	1.68	0.71	3.02	±0.73	<0.05	2.50	1.04	3.21	±0.69
Fructose-glucose ratio	1.58					1.28			

n: number of samples
a: expressed in dry matter
$\bar{x}$: mean value
min-max: range of measured values
s: standard deviation
P value: from Student's *t*-test

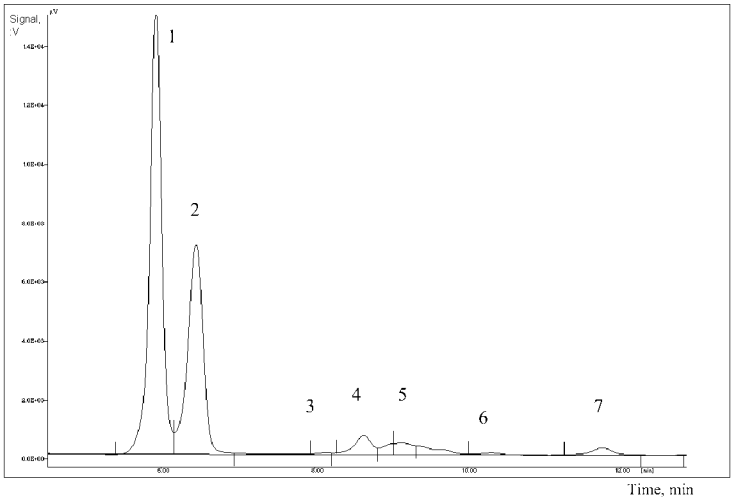

Fig. 1. Chromatogram of an robinia honey. Column: Supelcosil NH_2 (250×4.6 mm); mobile phase: acetonitril/water (75/25); flow rate: 1 cm^3 min^{-1}; column temperature: 35 °C; refractive index detector. Peaks: 1: fructose; 2: glucose; 3: saccharose; 4: turanose; 5: maltose; 6: isomaltose; 7: erlose + melezitose

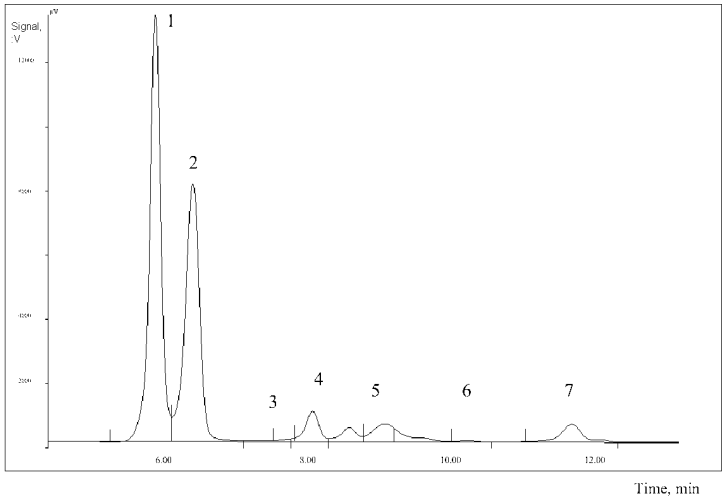

Fig. 2. Chromatogram of a milkweed honey. Column: Supelcosil NH_2 (250×4.6 mm); mobile phase: acetonitril/water (75/25); flow rate: 1 cm^3 min^{-1}; column temperature: 35 °C; refractive index detector. Peaks: 1: fructose; 2: glucose; 3: saccharose; 4: turanose; 5: maltose; 6: isomaltose; 7: erlose + melezitose

Saccharose, turanose and isomaltose were found in higher amounts, whereas maltose, melezitose and erlose in lower amounts in robinia than in milkweed honey. The turanose content of the robinia honey and the maltose content of the milkweed honey were outstandingly high and the sugar contents of the two unifloral honeys in the case of these two sugars were significantly different. We have to mention that the range of saccharose content was the highest both in robinia and milkweed honeys.

3. Conclusions

Based on our results, the conclusion can be drawn that milkweed honey is slightly darker in colour, more acidic and has higher enzyme activity values than robinia honey. The above-mentioned features will not spoil the quality of robinia honey if mixed with milkweed honey, but it is advisable to avoid mixing the two honeys, because the lower fructose-glucose ratio of milkweed honey makes the mixed robinia honey crystallize easier.

Robinia honey contained larger amounts of turanose and isomaltose and lower amounts of maltose than milkweed honey. This tendency is supported by the results of FÖLDHÁZI (1994) and SZABÓ (2001) as well. According to our studies, the total amount of erlose and melezitose content of milkweed honey is higher than that of robinia honey, similar to Szabó's results (SZABÓ, 2001). The differences between the sugar composition of the two unifloral honeys make possible to distinguish them.

References

FÖLDHÁZI, G. (1994): Analysis and quantitation of sugars in honey of different botanical origin using high performance liquid chromatography. *Acta Alimentaria*, *23*, 299–311.

GULYÁS, S. (1993): A selyemkóró (*Asclepias syriaca* L.) nektárjának és mézének összetétele homokon és kötött talajon. (Composition of milkweed nectar and honey on sand and hard ground.) *Méhészújság*, *6* (3), 10.

HUNGARIAN STANDARD (1980a): *Méz kémiai és fizikai vizsgálata. Savfok és pH meghatározása*. (Chemical and physical test of honey. Determination of acidity and pH.) MSZ 6943/3-80.

HUNGARIAN STANDARD (1980b): *Méz kémiai és fizikai vizsgálata. Víz-, illetve szárazanyagtartalom meghatározása*. (Chemical and physical test of honey. Determination of water and dry matter content.) MSZ 6943/1-79.

HUNGARIAN STANDARD (1981): *Méz kémiai és fizikai vizsgálata. Diasztáz-aktivitás meghatározása*. (Chemical and physical test of honey. Determination of diastase activity.) MSZ 6943/6.

KISS, T. (1983): A méz. (The honey.) -in: NIKOVITZ, A. (Ed.) *A méhészet kézikönyve*. (Handbook of apiary.) Állattenyésztési és Takarmányozási Kutatóközpont, Budapest, pp. 383–431.

KRAUZE, A. & ZALEWSKI, R.I. (1991): Classification of honeys by principal component analysis on the basis of chemical and physical parameters. *Z. Lebensmittelunters. –Forsch.*, *192*, 19–23.

MATEO, R. & BOSCH-REIG, F. (1997): Sugar profiles of Spanish unifloral honeys. *Fd Chem.*, *60*, 33–41.

PERSANO, O.L., BALDI, E. & ACCORTI, M. (1990): Diastatic activity in some unifloral honeys. *Apidologie*, *21*, 17–24.

SIEGENTHALER, U. (1977): Eine einfache und rasche Methode zur Bestimmung der Glucosidase (Saccharase) im Honig. *Mitt. Gebiete Lebensm. Hyg.*, *68*, 259–266.

SZABÓ, K. (2001): Mézek cukorösszetételének összehasonlító vizsgálata. (Comparative study of honeys' sugar components.) MSc thesis. Szent István University, Budapest, 66 pages.

SZÉL, ZS., KARDOS-NEUMANN, Á., BIACS, P.Á., SZALAI-MÁTRAY, E. & TAKÁTS, A. (2002): Investigation of enzyme activity in Hungarian acacia and milkweed honeys. *Acta Alimentaria*, *31*, 197–201.