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INVESTIGATION OF ENZYME ACTIVITY IN HUNGARIAN ACACIA AND MILKWEED HONEYS

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Diastase and glucose-oxidase activity was determined in 8 samples of Hungarian milkweed (*Asclepias syriaca*) honey and in 10 samples of Hungarian acacia (*Robinia pseudoacacia*) honey. The aim of the study was to compare diastase and glucose-oxidase activity of milkweed and acacia honey. Mean value for diastase was 16.28 diastase number (DN) (±2.53) in acacia honey and 24.48 DN (±5.07) in milkweed honey. Mean value for glucose-oxidase was 3.67 nmol unit g^{-1} (±3.31) in acacia honey and 8.24 (±4.21) in milkweed honey. The differences both in diastase and glucose-oxidase activities of the two honey types were statistically significant.

Keywords: acacia honey, diastase, glucose-oxidase, enzyme

Honey contains small amounts of different enzymes, the most important of which are diastase, invertase, glucose oxidase and catalase. The origin of these enzymes in honey is commonly attributed to the bee. The collected nectar is mixed with secretions from the salivary and hypopharyngeal glands of foraging bees. In the hive, when nectar is passed from bee to bee before stored in the cells, more secretions are added, enabling the nectar to ripen into honey (MAURIZIO, 1975).

Although enzyme content mainly originates from bees and partly from nectar, diastase activity in some unifloral honeys is consistently low (e.g. citrus honey) or high. The colour and consistence of acacia and milkweed honey are very similar. To distinguish acacia honey from milkweed honey by pollen analysis is impossible, because milkweed honey has no own pollen. We made an attempt to make a distinction between these two honey types by enzyme content.

The diastase enzyme, which converts starch to glucose, mainly consists of α-amylase and β-amylase. Diastase activities are parameters of honey quality control used as indicators of storage conditions and heating (WHITE, 1992).

Glucose-oxidase transforms glucose into gluconic acid and hydrogenperoxide. This transformation has antibacterial properties, but it is performed only in diluted honey.

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There are some factors which decompose hydrogenperoxide, e.g. catalase enzyme or ascorbic acid. The wound healing properties of honey are partly attributed to the presence of this enzyme.

1. Materials and methods

Diastase and glucose-oxidase activities were determined for 10 acacia (*Robinia pseudo-acacia*) and 8 milkweed (*Asclepias syriaca*) honeys. The milkweed samples originated from the middle part of Hungary and the Robinia honey from different areas of the country. Samples were extracted in summer of 1999 and they were stored under room temperature until February 2000.

Diastase activity was measured photometrically following Schade-White-Hadorn method (HUNGARIAN STANDARD, 1981) and expressed in DN (diastase number from Gothe). One diastase unit (DN) shows that how much 1% starch solution can be degraded in one hour at 40 °C by the diastase enzyme content of 1 g honey.

Glucose-oxidase was measured by Sigma method: modification of Bergmeyer method (ANON, 2000) and the enzyme extraction was carried out by three-phase partitioning method (SZAMOS, 1992). The activity of this enzyme is expressed in nmol unit g^{-1}.

Peroxide content of honey samples was measured by a semiquantitative procedure using a Merckoquant peroxide test strip (No. 110081, Merck, Darmstadt, Germany). The activity of glucose-oxidase is in direct ratio to the produced hydrogen peroxide. The obtained value, multiplied by five, gives the amount of hydrogen peroxide accumulation in micrograms per g honey per hour at 20 °C (KERKVLIET, 1996).

2. Results

Results of diastase and glucose-oxidase activity determinations in acacia and milkweed honey are presented in Table 1 and on Fig. 1 and 2. The average DN number in case of acacia honey was 16.28±2.53 that is close to the result (17.83±2.92) published by KEREKES and SITKEI (1996). The average DN number was 24.48±5.07 in the milkweed honey. Comparing the values of the diastase activity in acacia and milkweed honeys, statistically significant ($P<0.002$) differences were found.

The mean value of glucose-oxidase was 3.67±3.31 nmol unit g^{-1} in acacia honey and 8.24±4.21 in milkweed honey, and the difference was also statistically significant ($P<0.008$).

As both glucose-oxidase and diastase enzymes are produced in the pharyngeal gland of bee, the correlation between the DN number and the glucose-oxidase activity in the individual acacia and milkweed honey samples were computed statistically. The correlation coefficient was 0.38 in acacia honeys and 0.21 in milkweed honeys which are very low.

Table 1. Diastase numbers and glucose-oxidase activity in two honey types

Acacia honey				Milkweed honey			
Sample No.	DN number	Glucose-oxidase nmol unit g^{-1}	Peroxide test value	Sample No.	DN number	Glucose-oxidase nmol unit g^{-1}	Peroxide test value
1	15.35	1.16	3	1	21.66	7.72	3
2	15.00	2.35	1	2	22.16	16.35	10
3	20.35	0	0	3	19.86	3.40	3
4	15.89	1.15	3	4	34.11	12.82	10
5	19.73	9.66	10	5	26.90	0.36	10
6	19.65	8.70	10	6	18.63	6.15	3
7	14.73	1.22	1	7	28.03	6.80	3
8	16.04	4.10	3	8	24.52	6.67	3
9	14.05	3.31	3				
10	12.53	3.04	3				
min	12.53	0		min	18.63	3.40	
max	20.35	9.66		max	34.11	16.35	
$\bar{x}$	16.28	3.67		$\bar{x}$	24.48	8.24	
s	±2.53	±3.31		s	±5.07	±4.21	

min-max: range of measured values
$\bar{x}$: mean value
s: standard deviation

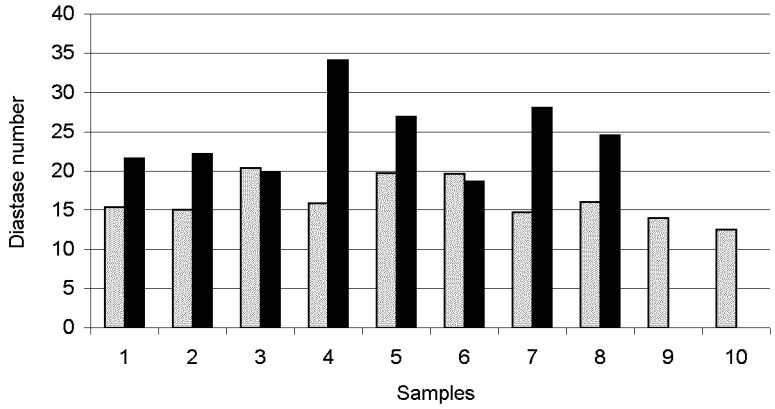

Fig. 1. Diastase enzyme activity in acacia and milkweed honey. ▨: Acacia honey; ■: Milkweed honey

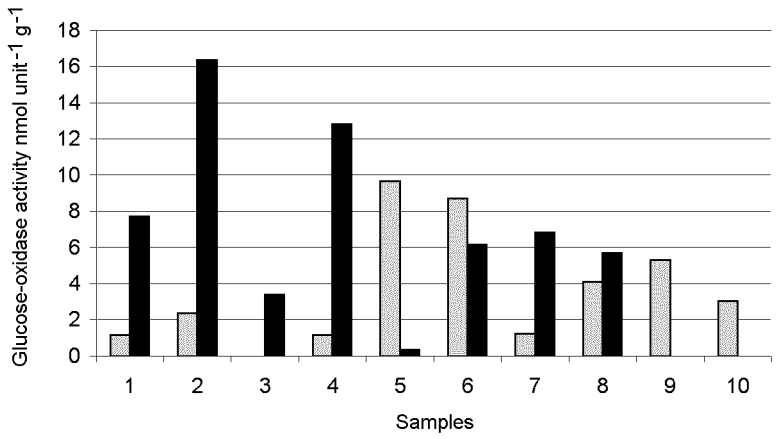

Fig. 2. Glucose-oxidase activity in acacia and milkweed honey. ▨: Acacia honey; ■: Milkweed honey

The glucose-oxidase values measured by Sigma method were compared with peroxide test values: the correlation coefficient was 0.92 in acacia honey and 0.38 in milkweed honey.

3. Conclusions

Milkweed honey has higher diastase and glucose-oxidase activity than acacia honey. Measuring enzyme content provides an opportunity to use this parameter as an additional index for distinguishing acacia and milkweed honey. These differences can be explained by different density of nectars. Milkweed nectar is probably thinner than

acacia nectar and bees need more time to concentrate nectar and add more enzyme to it. It can not be excluded either that the different enzyme content of bee saliva is induced by different nectars or the different enzyme content of variant nectars.

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