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PRELIMINARY ANALYSIS OF SOME LINDEN HONEY ORIGINARY FROM ARAD COUNTY

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ABSTRACT:
The honey is one of the most ancient energizing food and medicine used by the people. This miraculous product of the bees contain a lot of sugars and oligominerals that could help to complete the people diet, promote the health and give extra energy in case of exhausting activity, like those performing different sports. The aim of this study was to evaluate the linden honey originary from different location from Arad county for their quality, by determination of organoleptic properties, the sugars, calcium, magnesium, sodium, potassium and zinc, chloride and sulfates content. These determinations were performed using color standards by comparison, TLC respectively AAS. The results show high content of calcium, magnesium and zinc in all samples, contents that can improve the honey usage as functional food for supplement these essential oligominerals. It was observed that the sugars present in linden honey samples are mostly represented by fructose and glucose. The studied samples contain chlorides and sulfates in legal limits. All these preliminary results propose the Arad county linden honey as a valuable functional food for prevent different potencial diseases.

Keywords: Linden honey, sugars, oligominerals, TLC, AAS

INTRODUCTION:

Today, the honey is more used as a sweet food and less for its therapeutic effects even that in ancient times was considered a miraculous product made by bees. In last time it was a disputed product due by its origin, the bee products being considered potencial allergenic by a part, but from other point of view is more and more considered as a very important natural sweetener that can replace the sugar from some diets (Bauer et al., 1996). Studies had shown that the honey can have boosting effect on sport performance. The honey, as a source of carbohydrates, is important for produce energy, but also for recovery after the exhausting energy consumption. The trials show that honey consumption during the exercises and after energy consumption can improve the power, speed and endurance, sustains more longer the blood sugar level with a better stability of insulin level (Kreider, 2002).

In Arad county the bee-keepers have a long time history, due by the diversity of forester vegetation of this region. There are many locations from county in that the bees can collect the pollen from spring to late autumn due by the mild temperate weather with mediteranean influence. From this reason the bees can collect special pollens from that can be obtained the different kind of honey. One of the most popular is the linden honey due also by its diaphoretic, calming and mild emollient effects (http://www.bees-products.com/?p=articles&j=32).

Into the honey were identified more than 200 different bioactive compounds, from sugars to protides, vitamins, minerals, enzymes and not surprising also polyphenols with antioxidant activity. The honey contains mainly sugars, about 80 %, the main of them being the fructose (about 40 %) and the glucose (about 30 %) from class of monosaccharides and the sucrose from disaccharides. The main oligominerals from honey was found to be the calcium, iron, zinc, potassium and magnesium (da Silva et al., 2016; Mărghitaș, 2005).

The quality of the honey, generally, is given by national and international standards, but on the market can be find a lot of fake honey, with un-proper characteristics. In our country a lot of honey is sold directly by the bee-keepers. For this reason a study that can allow them to find the most proper area in that place the bees for a high quality honey is very important.

The aim of this study was to compare some characteristics of linden honey collected by individual bee-keepers from different region of Arad county. For this reason were evaluated the organoleptic properties, the sugars, calcium, magnesium, sodium, potassium and zinc, chloride and sulfates content.

MATERIALS AND METHODS:

The linden honey samples were collected from June to July 2016, from 3 regions: 2 samples from Ranusa (no. 2 and 8), 1 from Odvos (no. 13) and 1 from Dezna (no. 16). All samples are originary from individual bee-keepers that not use industrial processing of the honey. The samples were collected in standard, sterilized jars and were kept at room temperature.

The organoleptic analysis was performed by observation. The colour of the honey samples was compared with colour standards as are described in European Pharmacopoeia. There was used the BY colour scale.

The BY (brownish-yellow) solution is prepared from 2.4 parts of Y (yellow) solution, 1 part of R (red) solution, 0.4 parts of B (blue) solution and 6.2 parts of 10 g/L hydrochloric solution.

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The Y solution is prepared from 46 g ferric chlorid in 900 ml of a mixture (25 ml concentrated hydrochloric acid and 975 ml water) diluted at 1000 ml with the same mixture.

The R solution is prepared from 60 g cobalt chloride in 900 ml of a mixture (25 ml concentrated hydrochloric acid and 975 ml water) diluted at 1000 ml with the same mixture.

The B solution is prepared from 63 g copper sulfate in 900 ml of a mixture (25 ml concentrated hydrochloric acid and 975 ml water) diluted at 1000 ml with the same mixture.

The sugar analysis was performed by thin layer chromatography (TLC) using the conditions given by European Pharmacopoeia at Honey monograph. The analysis was performed on silica gel plate using acetonitrile and water (87:13, v/v) as mobile phase. 0.6 g of each honey sample was dissolved in 50 ml 30 % vol. ethanol and was applied 5 microliter as bands on the plate. As standards were used a mixture of 0.5 g fructose, 0.5 g glucose and 0.1 g saccharose in 100 ml 30 % vol. ethanol. It was applied also 5 microliter standard solution as band. After development on 15 cm the plate was sprayed with a reagent obtained from 2 g diphenylamine and 2 ml aniline in 100 ml acetone precipitate and dissolved with an appropriate quantity of 850 g/l phosphoric acid solution. Than the plate is heated at 100-105^{0}C for 5-10 minutes and vizualised in daylight.

The chloride and sulphate content was evaluated using the precipitation method and comparison with standard chloride and sulfate solutions as described in European Pharmacopoeia.

There were prepared 10 g/l respectively 40 g/l solutions from each linden honey samples.

15 ml of 10 g/l samples solutions was mixed with 1 ml 100 g/l nitric acid respectively 1 ml of 1.7 g/l silver nitrate solution. After 5 minutes the mixture is compared with 10 ml 5 ppm standard chloride solution and 5 ml water mixed with the reagents in the same way.

2.5 ml suspension (3 ml 250 g/l barium chloride and 4.5 ml 10 ppm standard sulfate solution) is mixed with 15 ml 40 g/l samples solutions, 0.5 ml glacial acetic acid. After 5 minutes the mixture is compared with 15 ml 10 ppm standard sulfate solution mixed with the reagents in the same way.

The calcium, magnesium, sodium, potassium and zinc content was evaluated by atomic absorption spectrophotometry (AAS) using a SensAA GBC Spectrophotometer (Australia). The analysis was performed with graphite furnace system with a lamp intensity of 5 mA, with a slit of 0.5 nm. The samples were injected with an autosampler that made a 0.1 dilution, the absorbtion and injection rates being 9 microliter/s and injection volume of 20 microliter. In table 1 are presented the standard solutions used for calibration curves and the curves equations. The initial solutions were prepared with ultrapurified water from 1000 mg/l standards purchased from Merck, Germany in 2-3 % nitric acid. As blank solution was used ultrapurified water. In table 2 are presented the wavelengths and the furnace temperature programs for each element.

Table 1.
The standard solutions used for AAS determinations

Element	Initial solutions concentration, ppm	Calibration curve range, ppm	Calibration curve equation	Correlation index
Sodium	20	0.25-2.00	A = 0.2401*c + 0.0559	0.9856
Potassium	20	0.50-2.00	A = 0.1420*c + 0.0330	0.9845
Calcium	20	0.50-2.00	A = 0.1843*c + 0.3202	0.9734
Magnesium	2	0.05-0.50	A = 0.6356*c + 0.1169	0.9709
Zinc	20	0.50-2.00	A = 0.1701*c + 0.4029	0.9752

Table 2.
The AAS graphit furnace conditions

Sodium, 586.60 nm					**Potassium, 766.50 nm**				
Stage	Final temperature, ^{0}C	Time, sec	Maintaining time, sec	Gas type	Stage	Final temperature, ^{0}C	Time, sec	Maintaining time, sec	Gas type
1	Sample injection				1	Sample injection			
2	85	5,0	3,0	Without	2	85	5,0	3,0	Without
3	95	40,0	3,0	Without	3	95	40,0	3,0	Without
4	120	10,0	3,0	Argon	4	120	10,0	3,0	Argon
5	700	5,0	3,0	Argon	5	700	5,0	3,0	Argon
6	700	1,0	3,0	Argon	6	700	1,0	3,0	Argon
7	700	2,0	0,0	Without	7	700	2,0	0,0	Without
8	2000	0,9	0,0	Without	8	2100	1,0	0,0	Without
9	2000	2,0	0,0	Without	9	2100	2,0	0,0	Without
10	2000	2,0	3,0	Argon	10	2100	2,0	3,0	Argon

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Calcium, 422.70 nm					Magnesium, 285.20 nm				
Stage	Final temperature, ^{0}C	Time, sec	Maintaining time, sec	Gas type	Stage	Final temperature, ^{0}C	Time, sec	Maintaining time, sec	Gas type
1	Sample injection				1	Sample injection			
2	85	5,0	3,0	Without	2	85	5,0	3,0	Without
3	95	40,0	3,0	Without	3	95	40,0	3,0	Without
4	120	10,0	3,0	Argon	4	120	10,0	3,0	Argon
5	1000	5,0	3,0	Argon	5	900	5,0	3,0	Argon
6	1000	1,0	3,0	Argon	6	900	1,0	3,0	Argon
7	1000	2,0	0,0	Without	7	900	2,0	0,0	Without
8	2600	1,2	0,0	Without	8	2200	1,0	0,0	Without
9	2600	2,0	0,0	Without	9	2200	2,0	0,0	Without
10	2600	2,0	3,0	Argon	10	2200	2,0	3,0	Argon

Zinc, 213.90 nm				
Stage	Final temperature, ^{0}C	Time, sec	Maintaining time, sec	Gas type
1	Sample injection			
2	85	5,0	3,0	Without
3	95	40,0	3,0	Without
4	120	10,0	3,0	Argon
5	300	5,0	3,0	Argon
6	300	1,0	3,0	Argon
7	300	2,0	0,0	Without
8	1900	0,8	0,0	Without
9	1900	2,0	0,0	Without
10	1900	2,0	3,0	Argon

RESULTS AND DISCUSSIONS:

The organoleptic observation of the samples shown that those from Ranusa present slight sugar deposits, more intensive is in sample no. 2. There is no significant changes in the color all samples being identical with the standard BY1, meaning a light brown-yellow color.

In figure 1 can be observed the TLC chromatogram of the four samples.

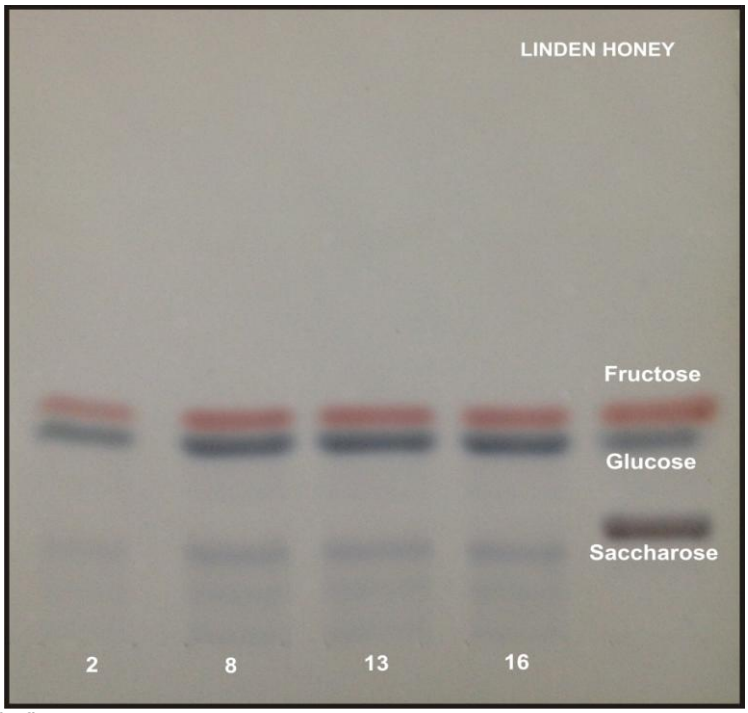

Fig. 1. The chromatogram in fluorescence

All samples contains high concentration of glucose and fructose and no saccharose. The samples contains also other compounds less the sample 2 in that could be identified just the two monosaccharides. From the relative intensity of the bands can be concluded that the samples from Ranusa are less concentrated in sugars. This can be explained by the partial precipitation of these compounds in time, being observed some deposits in these samples.

The chloride and sulfates must be maximally 350 ppm respectively 250 ppm according to European Pharmacopoeia. These ions were determined by precipitation with silver nitrate respectively barium chloride and the solutions were compared with standard chloride and sulfate solutions. All samples complies the European Pharmacopoeia requests. The intensity of turbing showing that the chloride content is slightly higher in sample 16 from Dezna. Sulfates exist in all samples but in lower concentration. The sulfate contents is very similar in all four samples.

In table 3 can be observed the oligominerals content in all four samples.

Table 3.
The content in oligominerals

Sample originary from	Content in, ppm				
	Sodium	Potassium	Calcium	Magnesium	Zinc
No.2 / Ranusa	648.4	451.3	833.4	209.0	81.5
No.8 / Ranusa	768.7	345.1	786.4	212.7	98.3
No. 13 / Odvos	714.2	1112.7	805.4	207.5	58.1
No. 16 / Dezna	667.8	928.0	803.9	210.8	116.5

It can be observed that the sodium level is between 648.4 and 768.7 ppm, the lower and highest levels being determined in the samples from Ranusa. The potassium level varying much, from 345.1 to 1112.7 ppm, the lowest level being in a sample from Ranusa and the highest in the sample from Odvos, on Dezna sample being also high. The calcium content is ranging from 768.4 to 833.4 ppm, both values being recorded in the samples from Ranusa. The magnesium content is 207.5 ppm in the sample from Odvos and not vary much, the highest value being recorded in the sample from Ranusa, 212.7 ppm. A high variation of values were observed at zinc content, from 58.1 ppm in Odvos sample to 116.5 ppm in Dezna sample.

USA database indicate as average value for minerals honey content 40 ppm for sodium, 520 ppm for potassium, 60 ppm for calcium, 20 ppm for magnesium respectively 2.20 ppm for zinc. In comparison, the mineral contents of the linden honey samples studied by us are much higher. The higher mineral content of Arad county's linden honey propose this bee product as a qualitatively superior food that can prevent osteoporosis by supplement the calcium, can be beneficial on heart and muscles functions due by the high content in magnesium and can sustain the good function of immune system and the activity of some essential enzymes by supplement the zinc. In same time some linden honey sorts (e.g. those from Odvos or Dezna) can supplement the potassium, an essential minerals for body homeostasis, at the persons with diuretic cures that can deplet the body of this element.

The higher content of linden honey in calcium and magnesium in case of the samples from Ranusa and Dezna can be explained also by the fact that these area are forest, mountain areas, and the forest soil is more rich in calcium and magnesium, being an ecological indicator of forest vegetation. Odvos is situated on Mures river plain, an area with intensive agricultural activity and contains more potassium, due probably also by the near cultures that could be fertilized with substances containing high potassium concentration. The higher zinc content in the samples from forest area can be explained also by the soil composition, the sandy soil, like those near rivers have lower zinc content as the high organic matter content forest soil.

CONCLUSIONS:

The study demonstrate the high alimentary and potencial therapeutic value of Arad county linden honey. The results show also the fact that the mineral composition of honey can be highly influenced by the soil composition, the area from where the bees collect the pollen for produce the honey.

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