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PHYSICO-CHEMICAL AND BIOCHEMICAL CHARACTERIZATION OF HONEY FROM BEAR GARLIC (*ALLIUM URSINUM*) FLOWERS FROM BIHOR COUNTY

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Abstract

Honey is a natural product of animal origin very important in human diet from many points of view. It is defined as "the sweet substance produced by honeybees from the nectar of blossoms or from secretions on living plants, which the bees collect, transform and store in honeycombs".
It plays an important part in our nutrition and it is well-known for its positive effects on health.
Allium ursinum L. (Ramson or wild garlic) is a perennial plant, widely distributed in Europe. The plant is valued by beekeepers, as well, since bear garlic flowers can serve as pollen and nectar sources for honeybees, completing the spring bee pasture.
In Bihor county, in the forest of Săldăbagiu grows wild bear garlic and a beekeeper from this county has managed to obtain honey from the flowers of this plant in 2016 and 2017. In this study, certain physico-chemical and biochemical parameters of honey from bear garlic from this region were investigated.

Key words: floral honey, wild garlic flowers, physico-chemical parameters, bioactive compounds.

INTRODUCTION

Honey is defined as "the sweet substance produced by honeybees from the nectar of blossoms or from secretions on living plants, which the bees collect, transform and store in honeycombs" (Codex Alimentarius Commission, 2002). Naturally, honey has been traditionally recognized as a valuable source of energy. It has also been recognized for its antimicrobial characteristics, possessed excellent antioxidant activity and is such a natural product with a number of therapeutic properties (Alvarez-Suarez et al., 2010; Basualdo et al., 2007, Bousaid et al, 2014).

It is a concentrated aqueous solution of invert sugar, that contains a mixture of other carbohydrates, amino and organic acids, minerals, aromatic substances, pigments, waxes and pollen grains to make it complex (Ajlouni and Sujirapinyokul, 2010; Manzanares et al., 2011; Rashed and Soltan, 2004).

Wild or bear garlic (*Allium ursinum* L.) is a perennial plant, widely distributed in Europe, occurring in various deciduous woodlands, preferring

damp shadow places, meso- and eutrophic, neutral to moderately acid soils of the hilly and the mountainous vegetation belt (Kovacs et al, 2007).
Allium ursinum L., has been used for centuries in folk medicine, food flavoring and as an ingredient for local dishes (Sapunjieva et al, 2012). In 1992 *Allium ursinum* was declared the "Medicinal Plant of the Year" by the Association for the Protection and Research on European Medicinal Plants.

The usable part of the plant is usually its delicate and fragile leaves, which should be collected in the first phase of blooming (Błażewicz-Woźniak and Michowska 2011), but Bear garlic has a rounded head of star-like flowers. In bear garlic the flowers are a clear white.

The plant is valued by beekeepers, as well, since bear garlic flowers can serve as pollen and nectar sources for honeybees, completing the spring bee pasture (Farkas et al, 2012).

Flowering in late April, early May, it provides an excellent spring bee pasture with good nectar flow. Its flowering period overlaps with oilseed rape (*Brassica napus)*, but the nectar flow is uneven and does not give as much as rape, even in favourable weather (Nagy, 2005).

Rate of nectar secretion was influenced by both, floral age and environmental factors, from which relative humidity was the most important, being significantly and inversely related to nectar production. Similarly, environmental factors were found to affect the nectar production of ramson, ranging from 0.16 to 0.42 mg nectar/flower/day, with an average of 52.13% sugar content (Peter, 1975). In their study, Farkas et al, 2012, sugar value was 0.14–0.25 mg in sunny weather, but remained below 0.1mg in changeable, cool weather.

The aim of this study is to investigate some physico-chemical and biochemical parameters of honey from bear garlic (*Allium ursinum*) flowers from Bihor county, in year 2016 and 2017.

MATERIAL AND METHOD

In Bihor county, in the forest of Saldabagiu grows wild bear garlic and a beekeeper from this county has managed to obtain bear garlic honey (BGH) from the flowers of this plant in 2016 and 2017.

A number of 6 honey samples were analyzed, 3 sample from each year. All samples were obtained directly from the same beekeeper of Bihor county (BGH 2016 and BGH 2017).

In each sample, we determined physico-chemical parameters: colour, water, ash, total acidity, HMF, and biochemical parameters: total sugar proline, total polyphenols (TP), total flavonoids (TF), antioxidant activity (AA) with FRAP method and the ascorbic acid content with spectrophotometric method.

Physico-chemical parameters

Physical-chemical parameters, like: colour, water, pH, acidity, ash, electrical conductivity, were analysed according to the Romanian Standard Analysis Methods (National Standard, 2009) and Harmonised methods of the IHC (Bogdanov, 2009), or with specific methods.

Colour - Since the colour of honey partly reflects the content of pigments with antioxidant properties (carotenoids, flavonoids, etc.), honey was diluted to 50% (w/v) with warm water (45–50°C), sonicated for 5 min and filtered to eliminate large particles.

Colour of honey samples was measured with 2 methods:

a. Colour intensity -ABS 450

The net absorbance ABS450, was defined as the difference between spectrophotometric absorbance at 450 and 720 nm (Shimadzu UVMini-1240 spectrophotometer), (Bereta, 2005)

$$ABS\ 450 = A450 - A720$$

b. Colour analysis with Pfund scale

The color of honey was determined by spectrophotometric measurement of the absorbance of a 50% honey solution (w/v) at 635 nm.

The honeys were classified according to the Pfund scale after conversion of the absorbance values (White, 1984).

$$mm\ Pfund = -38.70 + 371.39 \times Abs\ 635$$

where mm Pfund is the intensity of honey colour in the Pfund scale; Abs is the absorption of honey solution.

Water and total sugar - of the tested samples were determined with digital refractometer KRUSS model AR 2008.

pH – HATCH SensyION 378 multiparameter meter was used to measure the pH of a 10% (w/v) solution of honey prepared in ultrapure water.

Total acidity - by titration method (Bogdanov, 2009).

Ash content -The honey is ashed at a temperature no higher than 600°C and the residue weighed.

Electrical conductivity (EC) – Currently the measurement of electrical conductivity is the most useful quality parameter for the classification of honeys which can be determined by relatively inexpensive instrumentation. This has been confirmed by the data published by Persano Oddo and Piro, 2004. This parameter was measured for a 20 % (w/v) solution of honey suspended in ultrapure water, with HATCH SensyION 378 multiparameter meter.

HMF content was determined by spectrophotometric method (White, 1979). Each of the honey samples was divided into 2 clarified aliquots; water was added to one of the aliquots and absorption was read at λ=284 and 336 nm. This was compared to a second solution in which this absorption was eliminated by the addition of sodium bisulphate. Results were expressed in milligrams of HMF per kilogram of honey.

Biochemical parameters

Proline - The content of proline is defined as the colour developed with ninhydrin compared with a proline standard (SIGMA-ALDRICH, USA) and expressed as a proportion of the mass of honey in mg/kg. Proline and ninhydrin (REDOX, Romania) form a coloured complex. After adding 2-propanol (MERCK, Germany), the extinction of the sample solution and a reference solution at a 510 nm wavelength is determined (Bogdanov, 2009).

Extraction of antioxidant components - Antioxidant components from honey were extracted with water (10% solution) and with methanol/water mixture

Total polyphenols (TP) content was determined by using the Folin-Ciocâlteu (1927) colorimetric method developed by Singleton and Rossi (1965). A diluted extract (0.5 ml) or phenolic standard was mixed with 2.5 ml Folin- Ciocâlteau reagent and after 5 minutes 2.0 mL sodium carbonate (7.5%). The absorption was read after 2 h at 20°C, at 750 nm. For the preparation of calibration curve 0.5 ml aliquot of 0.2, 0.4, 0.8 and 1.2 μM/ml aqueous gallic acid solution were used as the standard and expressed as mg of gallic acid equivalent (GAE).

Total flavonoids (TF) The total Flavonoid compounds content was measured with $AlCl_3$ colorimetric assay (Atanassova et al, 2011). The absorbance was measured at 510nm. As standard we used quercitine.

Ascorbic acid content was determined through xylene-extraction (Ranganna, 1986) using a spectrophotometer.

Antioxidant activity – FRAP assay (Berzie and Stain, 1996) using the calibration curves for $FeSO_4 \cdot 7H_2O$ (0 to 1000 μM) and Trolox (0 to 250 μM. Results were expressed as the correspondent of $FeSO_4x7H_2O$ and Trolox activity for 10% honey solution activity.

RESULTS AND DISSCUSIONS

After performing the calculations, it was found that in the analyzed honey samples in both years the colour was Extra light amber (table 1).

The honey samples had fluid consistency, was homogeneous, viscous, without signs of crystallization, without impurities. The flavor is of bear garlic, medium sweetness and, sometimes, a light bitterness.

The colour of honey was determined with 2 methods.

Table 1.

Mean values of the results obtained after colour intensity determination of the honey samples from the two years of study

Sample	Abs 450	Abs720	A450	Colour	A635	Pfund	Colour
BGH 2016	0.758	0.259	0.499	extra light amber	0.211	39.77	extra light amber
BGH 2017	0.834	0.268	0.566	extra light amber	0.235	48.57	extra light amber

The cotent of water, total sugar, ash, electrical conductivity total acidity and HMF respect the limits established for honey in the Romanian and the International Regulatory Standards.

Proline content was higher than 200 mg/kg honey, which proves that no sugars were added to the analyzed samples (table 2.).

Table 2.

Mean values for physical-chemical parameters determination

Sample	Acidity °T	Total sugar %	Water %	Electrical conductivity mS/cm	Ash %	Proline mg/kg	HMF mg/kg
BGH 2016	3.2	81,5	16,9	0.136	0,1	387.6	1,40
BGH 2017	3.5	79.2	19.2	0.158	0,17	303.2	1.20
STAS* 784/ 3-89/	**Max 4**	**Max 83**	**Max 20**	**0.8**	**Max 0,5**	**Min 200**	**Max 1,5**

The results obtained after performing analysis for studying the content of some bioactive compounds in honey samples from both years were inserted in table 3 and figure1.

Since no studies were found for bear garlic honey characterization, the data obtained by us were compared with those obtained for the acacia honey and the multifloral honey from April-May.

Table 3.

Sample	Total polyphenols mg GAE/kg honey	Flavonoids mg Quercitin/100g honey	FRAP value for a 10% honey solution (µmol $FeSO_4$)	Vitamina C mg/100g honey
BGH 2016	46.7±0.9	8.9±0.1	335.5±1.3	8.65±0.4
BGH 2017	38.65±0.8 ***	7.5±0.1 ***	309.0±1.1 ***	7.16±0.3 **

The total phenolic content of the tested honey samples, was 46.7 GAE/kg for year 2016, and 38.65 GAE/kg for 2017. These values are lower than results obtained for accacia and multifloral honey, by Beretta et al, 2005 (55.2±2.8 mg of GAE/kg of honey). Similar results were obtained by Bertoncelj et al., 2012, (from 25.7 to 67.9 mg of GAE per kg of honey) and Mărghitaş et al., 2009, who determined the total phenolic content in Romanian acacia honey to be in the range from 2.0 to 39.0 mg of GAE per kg of honey.

Total Flavonoid content has similar patern with those determined by Meda et al. (2005) in various Burkina Fasan honey types in the range from 0.17 to 8.35 mg of QE per 100 g of honey.

Antioxidant activity was 335.5 µM $FeSO_4$ in 2016 in comparison with 309 µM $FeSO_4$ in 2017, and similar with results obtained by Saric et al 2012.

Ascorbic acid content had close values for the two years, respectively 8.65 and 7.16 mg/kg of honey.

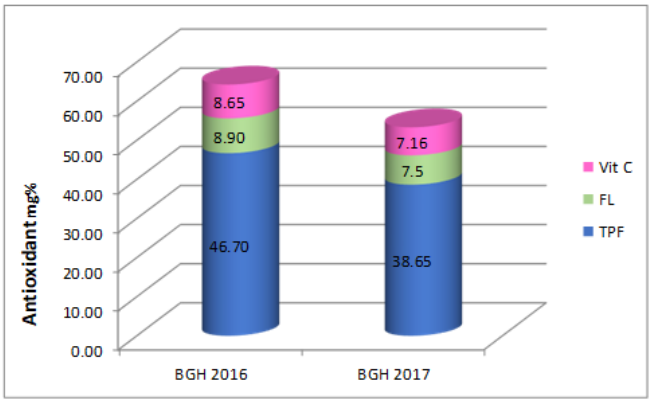

Fig.1 Graphical representation of mean values of vitamin C, total polyphenol and flavonoid content in the studied bear garlic honey samples

CONCLUSIONS

Bear garlic honey colour was extra light amber.

Physical-chemical parameters of bear garlic honey respect the limits established for honey in the Romanian and the International Regulatory Standards.

The studied antioxidant compounds content in Bear garlic honey has similar values like multifloral honey collected in April-May.

All the tested honey samples present a good quality, consistent with the legislative requirements and a high antioxidant activity.

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