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THE ANTIOXIDANT PROPERTIES AND MICROBIOLOGICAL QUALITY OF POLISH HONEYS

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ABSTRACT

Twenty samples of honey were collected from Polish apiaries and examined to antioxidant activity (FRAP method) as well as total phenolic compounds content. Moreover analysis of microbiological contamination of honeys with bacteria and fungi was conducted which was compared with water content and water activity. The highest antioxidant activity was detected for buckwheat honey and the lowest for the rape and acacia honey. This parameter was positively correlated (r=0.89) with the phenolic compounds content. The total number of bacteria and microscopic fungi in Polish honeys varied widely ranged to 2.4 - 4.59 and 0 - 3.93 log cfu/g, respectively. Microbiological contamination was independent on honey variety as well as its water activity. However, the antioxidant activity of honey was negatively correlated (r=-0.35) with total number of fungi what means that antioxidants inhibit their growth in honey.

Keywords: honey, antioxidant activity, phenolic compounds, bacteria, fungi

INTRODUCTION

Honey, like most natural products can be very diverse in terms of biologically active components. This depends largely on its origin. Best biological properties have floral honeys, an example is the Manuka honey from New Zealand which is considered as a medicine. It has been shown that the composition of honey depends largely on plant material from which it is derived. Therefore, the origin of honey, can determine its antibacterial properties.
The quality of the honey is mainly determined by its sensorial, chemical, physical and microbiological properties. On the other hand, in EU legislation there are no data about the microbial contamination and hygiene of products. In fact, around the world there have been several reports about the physicochemical parameters, however, microbial contamination studies are very rare and are mostly devoted to *Clostridium botulinum* (**Kačániová *at al.*, 2011**). There are several sources of microbial contamination of honey. The first group of pollutants include pollen, the digestive tract of bees, dust, air, soil and nectar, and these factors are very difficult to eliminate. The second group of pollutants refers to skills and qualifications of beekeepers and further processing of honey. These contaminants are easier to eliminate through the use of good manufacturing practices. This group of pollutants include molds and yeasts, and germ *Bacillus spp* and *Clostridium spp*, which are indicators of the quality and safety of commercial honeys (**Snowdon and Cliver, 1996**).
On the other hand honey has a bacteriostatic and bactericidal activity against several human pathogens. It is one of the most important not only healthy but also nutrition characteristics of honey (**Olaitan *et al.*, 2007**). Antibiotic properties of honey depend on many factors, chemical as well as physicochemical properties. One of them is a high osmotic pressure, which is the result of high concentrations of sugars, averaging about 77%. A high concentration of sugar in combination with a low moisture content causes osmotic stress which prevents honey's spoilage by microorganisms. Numerous studies have shown contradictory results concerning the impact of low pH (4.1 on average) on the antibacterial activity of honey (**Kwakman and Zaat, 2012).** Honey contains also the chemical substances which are largely responsible for the antibiotic activity of honey. Among the many components of honey, next to hydrogen peroxide and enzymes, also substances with antioxidant activity may play an important role in the overall antibacterial activity of honey. Therefore, to evaluate the content of these components in different types of honey can provide information on their healthy activities (**Hołderna-Kędzia and Kędzia, 2006**).
The aim of this study was to evaluate the quality of Polish honey in terms of antioxidant nutrient content and microbiological purity as well as to determine the effect of honey variety on the content of antioxidants and microbiological purity of honey.

MATERIAL AND METHODS

Honey samples (n=20) which were collected in the season 2011-2012 (Table 1) were taken directly from beekeepers in the South-eastern Poland. Honeys were collected at different times and stored at room temperature until analysis. Among analyzed samples there were 13 light and 7 dark honeys (colour was determined visually).

Water content

The water content was determined in triplicate using a portable refractometer (Atago ®, Tokyo, Japan).

Water activity

The water activity of each sample was measured by LABMASTER-AW (Novasina, Pfaffikon, Switzerland). Each sample was analyzed in three parallel determinations.

Antioxidant activity

To determine the total antioxidant activity of honey FRAP method was used (**Piljac-Zegarac *et al.*, 2009**). The 2,4,6-Tris (2-pyrimidyl)-s-triazine (TPTZ, Sigma, USA) was used. Standard curves was prepared for the ethanol solution of Trolox (Sigma, USA). The total antioxidant capacity (TAC) was calculated in Trolox equivalents [µmol Trolox/kg of honey].

Phenolic compounds

Determination of total phenolic compounds in honey based on its reaction with Folin-Ciocalteu reagent (Merck, Germany) according to **Piljac-Zegarac *et al.* (2009**). A standard curve was prepared for the solution of gallic acid (Sigma, USA). The total content of phenolic compounds was calculated in gallic acid equivalents (GAE) [mg of gallic acid/kg of honey].

Microbial contamination

For the microbiological analysis dilution plating method was applied. Sample of 5 g of honey were mixed with 45 cm^3 of saline (0.85% NaCl) and homogenized for 30 min (dilution 10^{-1}). Then, in accordance with the principle of decimal dilutions of the solution 10^{-2} was prepared. Bacteria were grown in Petri dishes on a substrate GTK at 30 ^{0}C for 48-72 hours. Microscopic fungi were growing on substrate DRBC at 25 ^{0}C for 5 days. After incubation all plates were analyzed for the appearance of bacteria and microscopic fungi. Number of microorganisms were presented as log colony forming units per gram of honey (log cfu / g).

Statistical calculations

The comparison between light and dark honeys were done with t-Student test. The Pearson correlation coefficient was calculated. All calculations were made using Excel 2007.

RESULTS AND DISCUSSION

Chemical properties of tested honey are summarized in Table 1.

Table 1 The results of water and antioxidant examinations in tested honeys

Sample		Year	Water content [%]	Water activity [a_w]	Antioxidant activity [µmol trolox/kg]	Phenolic compounds [mg GAE/kg]
LIGHT HONEY						
1	Rape (*Brassica napus*)	2011	19.7	0.566	455.48	105.64
2	Rape (*Brassica napus*)	2012	19.7	0.558	367.58	96.42
3	Lime (*Tilia*)	2011	16.1	0.569	573.06	151.74
4	Lime (*Tilia*)	2012	18.6	0.563	619.86	199.42
5	Sunflower (*Helianthus L.*)	2012	15.5	0.506	535.39	167.02
6	Willow (*Salix L.*)	2011	15.4	0.502	759.13	163.33
7	Willow (*Salix L.*)	2012	17.3	0.517	624.43	157.53
8	Goldenrod (*Solidago virgaurea*)	2011	15.8	0.526	874.43	199.68
9	Goldenrod (*Solidago virgaurea*)	2012	18.7	0.564	611.87	183.61
10	Phacelia (*Phacelia Juss.*)	2011	21.1	0.622	936.07	178.61
11	Phacelia (*Phacelia Juss.*)	2012	20.2	0.597	441.78	150.68
12	Acacia (*Acacia Mill.*)	2011	13.2	0.462	716.89	155.16
13	Acacia (*Acacia Mill.*)	2012	16.6	0.539	347.03	126.45
DARK HONEY						
14	Honeydew	2011	17.0	0.558	1206.62	263.44
15	Buckwheat (*Fagopyrum esculentum*)	2011	17.8	0.556	1632.42	430.98
16	Buckwheat (*Fagopyrum esculentum*)	2012	18.7	0.547	1235.16	432.03
17	Heather (*Calluna vulgaris*)	2011	18.9	0.596	1590.18	391.99
18	Heather (*Calluna vulgaris*)	2012	18.9	0.583	901.83	341.94
19	Multifloral	2011	16.8	0.556	1051.37	245.79
20	Multifloral	2012	17.4	0.526	576.48	204.43

Tested honeys were characterized by diverse water content and water activity (Table 1). The percentage of water in tested Polish honeys ranged from 13.2 to 21.1%, and the mean value was 17.7%. In 50% of the samples of honey water content was lower than average. 90% of samples tested showed a water content of less than 20%, which is below the maximum amount permitted by the EU (**Finola *et al*., 2007**). Similar results were determined for Slovak honey, where the water content within the range 14.13-20% was observed (**Kačániová *et al*., 2007**). The Indian honeys water content ranged 17-21.4% (**Iurlina and Fritz, 2005**).

The water activity of the tested Polish honeys ranged from 0.462-0.622 (Table 1), and the mean of 20 samples was 0.551. Only 5% of the samples posses the activity higher than 0.60. These findings are in agreement with other reports of water activity examination in Slovak honeys (0.460-0.660) and Polish honeys (0.509-0.608) (**Kačániová *et al*., 2007; 2012**). Slovenian honeydew honeys analyzed by **Abramovic *et al***. (**2008**) showed the water activity in the range 0.483 to 0.591. Moreover, in flower Slovenian honeys water activity ranged from 0.479-0.557 (**Abramovic *et al*., 2008**).

The lowest antioxidant activity of the studied Polish honey was found in rape honey (411.53 mmol/kg on average) (table 1). Antioxidant activity of Polish honey increased in the following order: acacia, sunflower, lime, phacelia, willow, goldenrod and multifloral. High antioxidant activity showed heather and honeydew honey, their activity amounted to 1206.62 and 1246.00 mmol/kg of honey, respectively. The highest antioxidant activity showed a buckwheat honey where their average activity of two samples was 1433.79 mmol/kg. The mean value of antioxidant activity for dark honeys was significantly ($P<0.001$) higher for dark honeys in comparison to light honey varieties (Fig. 1). These findings are in agreement with **Kesic *et al.* (2009)** observations, where the levels of antioxidants measured by FRAP method ranged from 95 to 2705 mmol/kg. They observed the weakest activity in acacia honey (335 mmol/kg on average), the middle level in multifloral honeys (1156.5 mmol/kg) and the highest in forest honey (1933.75 mmol/kg).

The highest amount of phenolic compounds in Polish honeys showed buckwheat honey (mean 431.51 mg/kg), followed by heather (366.97 mg/kg) and honeydew (263.44 mg/kg), while the lower content were found in rape honey, an average of 101.03 mg/kg (Table 1). The average content of these compounds in the light honey was significantly ($P<0.001$) lower, by 2-times in comparison with dark honeys (Fig 1). The content of phenolic compounds in the tested honey was very close to the results received by **Beretta *et al.*** (**2005**). The lowest content of these compounds, they found in acacia honey (55.2 mg/kg) whereas dark honeys showed the highest values 170.4 to 255.6 mg/kg for honeydew and 482.2 mg/kg for buckwheat honeys, respectively.

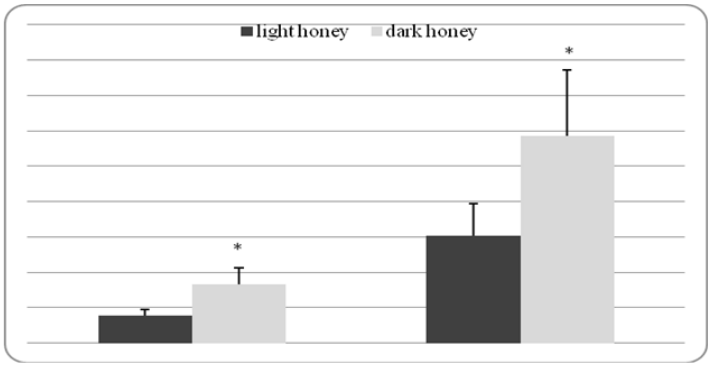

Figure 1 The comparison of antioxidant properties of light (n-=13) and dark (n=7) tested Polish honeys. (*) significant differences ($P<001$)

Botanical origin of honey has a very large impact on its antioxidant activity, while the processing, handling and storage has a less effect (**Al-Mamary *et al*., 2002**). It was also found out that the dark-colored honey, such as multifloral, buckwheat, heather and honeydew honeys have higher total phenolic content and, consequently, a higher antioxidant capacity than light honey such as rapeseed and acacia (**Bertoncelj *et al*., 2007**). It has been also shown that the compounds of the group of antioxidants, such as phenolic compounds are capable of inhibiting the growth of many Gram-positive and Gram-negative bacteria (**Kačániová *et al*., 2011**).

The results obtained for Polish honeys showed that the dark-colored honeys are characterized by a higher content of compounds as antioxidants. Honey with a high content of phenolic compounds was characterized by high antioxidant activity. The content of phenolic compounds in the investigated honeys was positively correlated with FRAP test results (Table 2). These data confirm that phenolic compounds are responsible for the antioxidant properties of honey.

Table 2 The correlation between the all tested parameters of Polish honeys

	Phenolic compounds	Antioxidant activity	Water content	Water activity	Total number of bacteria
Phenolic compounds	1				
Antioxidant activity	0.8918	1			
Water content	0.1338	0.0471	1		
Water activity	0.2301	0.2193	0.8593	1	
Total number of bactera	-0.1026	-0.0137	0.0169	0.2015	1
Total number of fungi	-0.3467	-0.3290	-0.0025	0.1383	0.1649

The total number of bacteria in tested honeys samples was close the range 2.40-4.59 log cfu/g, with an average of 3.50 log cfu/g (Fig 2). In 50% of the samples the contamination was above the average number of bacteria (Fig. 2). Research conducted on the Slovak honeys by **Knazovicka *et al.* (2010)** showed close relation of total number of bacteria to our research, which amounted to 1.38 log cfu/g of raspberry honey, and 3.14 log cfu/g of forest honey. Studies carried out on the Nigerian honey by **Ayansola and Banjo (2012)** showed a slightly higher total number of bacteria, which ranged from 3.00-6.20 log cfu/g. Results for Argentinean honeys published by **Iurlina and Fritz (2005)** showed lower total number of bacteria from 1.79 log cfu/g to 3.04 log cfu/g.

Number of microscopic fungi in samples of Polish honeys ranged from 0 to 3.93 log cfu/g, with a mean value of 1.42 log cfu/g (Fig. 2). The greatest contamination with the fungi was detected in lime honey whereas the fungi were not present in honeydew honey. A study published by **Felsociova *et al.* (2012)** conducted in Polish honeys confirmed our results. Authors discovered the number of fungi ranged from 0 to 2.00 log cfu/g and in 40% of samples they did not noticed these microorganisms. The maximum number of microscopic fungi in Argentine honeys was received as 2.00 log cfu/g (**Finola *et al.*, 2007**). On the opposite, analyses conducted on the Nigerian honeys by **Ayansola and Banjo (2012)** showed higher amounts of microscopic fungi than in Polish honeys. For the 18 samples available in the local market in the north-western Nigeria, authors found the presence of fungi at the level of 3.00-6.20 log cfu/g.

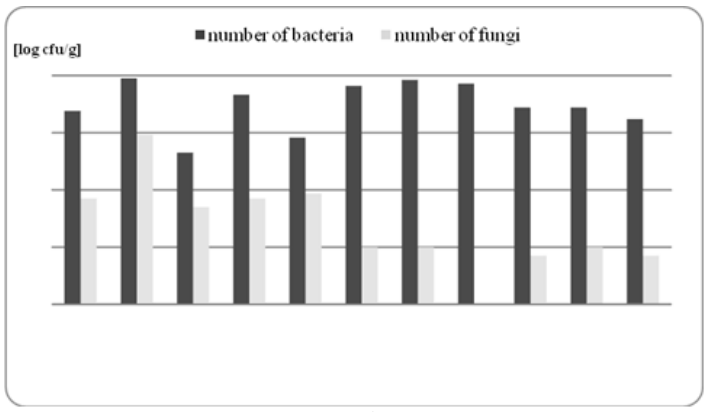

Figure 2 Microbiological contamination of tested Polish honeys.

There was no difference (P>0.05) between light and dark honeys in term water activity as well as total number of bacteria (Fig. 3). Light honeys showed higher contaminations by fungi than dark varieties (P<0.05).

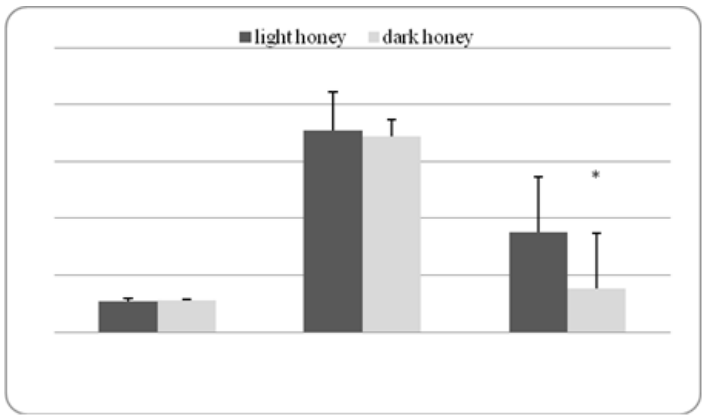

Figure 3 The comparison of light (n=14) and dark (n=6) tested Polish honeys. (*) significant differences (P<0.05)

The quality of honey also determines its microbiological profile, but in European and Polish legislation does not set out fully and unequivocally standards, which honey should have in terms of contamination by microorganisms. The honey industry recognizes that water is a key factor for the process of spoilage of honey. However, no absolute water content, but the water activity (a_w) in the honey controls the growth of bacteria. It is an important factor in preventing or reducing microbial growth. In addition, the water activity is the primary parameter responsible for the stability of honey, modulating microbial reaction and determining the type of microorganisms present in honey (**Abramovic *at al.*, 2008**).

Several studies have shown that the water content of honey is highly correlated with the activity of water in the product (**Chirife *et al.*, 2006**). This thesis is confirmed by the own study, where the correlation between water content and water activity in the Polish honeys was r = 0.8593 (Table 2). Furthermore it is assumed that the water activity is responsible for the stability, growth, and types of microorganisms present in honey. The analysis based on the total number of bacteria and fungi in the studied Polish honeys showed no significant correlation between these parameters (Table 2). However, the antioxidant activity of honey was negatively correlated (r= -0.35) with total number of fungi what means that antioxidants inhibits their growth in honey. The results of the statistical analyzes seem to indicate that the tested microbial contamination of honey is rather the result of the origin of honey and the method of its production, and not the botanical origin.

From the point of view of consumer health security, occurrence of anaerobe sporulating bacteria, including *Clostridium botulinum*, may have some significance. In normal fresh honey, aerobic bacteria are found in a small number and their high number indicates a contamination of honey from secondary sources. These organisms theoretically should not grow in honey but some of them may persist in it. Similarly, the number of yeasts and moulds in fresh honey is usually low but under some conditions these organisms are able to multiply in honey during its storage. These organisms may cause fermentation of honey during storage, which may be a significant economic problem. Results obtained by **Różańska and Osek** (**2012**) for Polish honeys samples during one year storage confirmed the opinions that microorganisms are not able to multiply in honey. Only in a low number of samples, the increasing number of aerobes (for 13% of samples), molds and yeasts (15%), and growth of anaerobe spore forming bacteria (16.5%) was noted.

CONCLUSION

Among tested honey the highest antioxidant activity was observed for buckwheat honey, heather and honeydew, the lowest for the rape and acacia honey. The total antioxidant capacity was positively correlated with the content of phenolic compounds. The total number of bacteria and microscopic fungi in Polish honeys varied widely ranged to 2.4-4.59 and 0-3.93 log cfu/g. The water content in the test was significantly correlated with the activity of water, but these parameters do not affect the level of microorganisms in honey.

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