Emirates Journal of Food and Agriculture. 2018. 30(12): 990-997
doi: 10.9755/ejfa.2018.v30.i12.1864
http://www.ejfa.me/

REGULAR ARTICLE

Effect of honey supplementation with bee products on quality parameters and mineral composition

Lesław Juszczak*, Adam Florkiewicz, Robert Socha, Dorota Gałkowska, Anna Piotrowska

Department of Food Analysis and Evaluation of Food Quality, Faculty of Food Technology, University of Agriculture in Krakow, Balicka 122, 30-19 Krakow, Poland

ABSTRACT

The aim of this work was to evaluate the influence of supplementation of multiflower honey with bee products on selected quality parameters and on mineral composition. Supplementation of honey with bee products slightly affected the water content; however, part of samples had slightly exceeded the allowed water content. Addition of individual bee products resulted in significant increases in the amount of insoluble solids, free acidity and conductivity. The greatest effect was observed for honeys with pollen or beebread, due to higher levels of supplementation of honey with these products. The addition of bee products had little effect on HMF content. Supplementation of honey with beebread resulted in lower glucose and fructose content. Honeys with bee products exhibited slightly higher sucrose content, while in three of the analyzed samples sucrose content exceeded the allowed for honey limit. Supplementation of honeys with pollen or beebread significantly influenced an increase in K, Ca, Mg, Fe, Zn and Mn contents.

Keywords: Honey; Propolis; Pollen; Bee bread; Quality parameters; Minerals composition

INTRODUCTION

Bee products are valuable components of human diet. They are rich source of biologically active compounds, of which the preventive and therapeutic properties are determined by amount of very diverse groups of compounds (Juszczak et al., 2016; Socha et al., 2016). Bee products can be also attractive health promoting food ingredients and components of functional food (Viuda-Martos et al., 2008). They are used for centuries in traditional folk medicine. They exhibit a very broad spectrum of antibacterial, antivirus, antiradical, antioxidant and anticancer properties and are used in prevention and treatment of many diseases (Viuda-Martos et al., 2008; Rodriguez et al., 2012; Jamróz et al. 2014, Habib et al, 2014; Juszczak et al., 2016). Rady et al. (2018) observed that non cytotoxic Sidr Kashmiry honey could be a powerful pro-apoptotic and non-genotoxic anticancer agent. Also, Afrin et al. (2018) stated that Manuka honey could be a useful preventive or adjuvant agent in the treatment of colorectal cancer. In turn, Gasparrini et al. (2018) concluded, that Manuka honey acted as a natural agent for preventing oxidative and inflammatory-related diseases.

The qualitative parameters of honey as a natural and valuable product are strictly regulated by the relevant legislation, and the product that does not meet them should not be in a trade. Beside commonly consumed honey, the valuable bee products include also royal jelly, pollen, beebread and propolis (Viuda-Martos et al., 2008, Ramadan and Al-Ghamdi, 2012; Isidorov et al., 2009).

Royal jelly is extremely valuable product produced by young bees to feed the larvae of worker bees and queen bees. It contains all the essential amino acids and it is valuable source of enzymes (Ramadan and Al-Ghamdi, 2012). One of the most important bioactive properties of royal jelly is positive influence on the cardiovascular system by regulating blood pressure. On the one hand, royal jelly enlarges blood vessels and lowers blood pressure thanks to the presence of acetylcholine, and, on the other hand, it increases blood pressure by increasing the secretion of adrenaline. Royal jelly has also antiatherogenic effect due to the lowering the level of cholesterol in blood. It is used in the treatment of anemia, because it increases the number of red blood cells and hemoglobin content and increases the concentration of iron in blood (Viuda-Martos et al., 2008, Ramadan and Al-Ghamdi, 2012).

***Corresponding author:**
Lesław Juszczak, Department of Food Analysis and Evaluation of Food Quality, Faculty of Food Technology, University of Agriculture in Krakow, Balicka 122, 30-19 Krakow, Poland, **Phone/fax:** +48 12 6624746, **E-mail:** rrjuszcz@cyf-kr.edu.pl

Received: 28 April 2018 ; **Accepted:** 29 November 2018

Pollen is composed of collection of male reproductive cells of seed plants, formed in the anthers of flowers in the form of grains. It provides food for bees and larvae (Hongcheng et al., 2015). Especially valuable component of pollen is arginine – a conditionally essential amino acid, because it stimulates the production of nitric oxide which reduces blood vessel wall tension and thus it shows diastolic action. Very important group of bioactive compounds present in pollen are flavonoids with predominating flavanols (Hongcheng et al., 2015). In order to ensure the proper amount of food, bees produce a beebread, which is a mixture of pollen, honey and bee secretions of the salivary glands. Under anaerobic conditions, it is subjected to fermentation under the action of enzymes contained in the saliva of bees and bacteria of the *Lactobalillus genus*. Through the fermentation process the pollen becomes better digestible as compared to the bee pollen, because the nutrients present in it undergo significant chemical changes (Isidorov et al., 2009). Wide chemical composition of bee pollen and beebread determines their diverse biological properties (Isidorov et al., 2009; Llnskens and Jorde, 1997). Unsaturated fatty acids, phospholipids and phytosterols present in these products exhibit hypolipidemic activity. Phospholipids show also lipotropic properties, because they prevent the formation and deposition of lipids in a liver. Other biologically active compounds are formed from the unsaturated fatty acids, e.g. prostaglandin and prostacyclin, which have relaxant acting on blood vessels and reduce blood platelet aggregation, thus preventing blood clots (Llnskens and Jorde, 1997). In addition to the antiradical and antitumor activity, the phenolic acids and flavonoids present in pollen and beebread have a number of other functional properties and are also valuable sources of vitamins and bioelements (Llnskens and Jorde, 1997; Orzáez Villanueva et al., 2001; Socha et al., 2018).

Propolis is a substance produced by bees from resinous-waxy secretion of plants. (Sahinler and Kaftanoglu 2005; Farooqui and Farooqui 2012). The biological activity of propolis is due to its chemical composition and interactions of the individual components (Viuda-Martos et al. 2008; Farooqui and Farooqui 2012). Propolis contains mainly flavonoid aglycones of flavones, flavonols, flavonones and chalcones (Sahinler and Kaftanoglu 2005; Socha et al., 2015). Phenolic compounds present in propolis affect the inhibition of formation of reactive oxygen species (Farooqui and Farooqui 2012). Antioxidant properties of propolis determine also its anticarcinogenic activity, which manifests in inhibition of the proliferation of cancer cells and causing apoptosis. The most important compound in the prevention of cancer is CAPE – caffeic acid phenethyl ester, however, the prerequisite for its activity is synergistic effect of all anticancer components of propolis (Farooqui and Farooqui 2012).

Bee products gain more and more appreciation of consumers due to presence of bioactive compounds. The most natural way of introducing bee products such as royal jelly, pollen, propolis and beebread to the human diet seems to be addition them to a honey. Therefore, the aim of this work was to assess the effect of addition of bee products to natural honeys on selected quality parameters and mineral composition.

MATERIALS AND METHODS

Materials

The materials used were commercial samples of multiflower honeys supplemented with various bee products: royal jelly, beebread, propolis and pollen. Twenty two honey samples, which were obtained from five producers were analyzed. The honeys were obtained from the following apiaries of the southern Poland: Apiary "Barć Kamianna", Łabowa, Lesser Poland, Apiary "PH Barć", Biszcza, Lublin Province, Beekeeping Farm "Sądecki Bartnik", Stróże, Lesser Poland, Apiary "Pasieka pod Pilskiem", Korbielów, Silesia, Beekeeping Farm "Łysoń", Sułkowice, Lesser Poland. The honeys from each of these apiaries were multiflower honeys and honeys supplemented with various bee products, however, not each manufacturer offered all the kinds of supplemented samples therefore, the number of samples in each group varies.

Methods

Water content was determined by refractometric method using Reichert-Jung Abbe Mark II refractometer (USA). Insoluble solids content was determined by weight method. Free acidity was determined by titration of honey with 0.1 M NaOH to pH = 8.3. Electrical conductivity of honey solutions was determined by conductometric method using CPC-501 conductometer (Elmetron, Poland). The 5-(hydroxymethyl)furfural content was determined by reversed-phase high performance liquid chromatography with spectrophotometric (UV) detection. The LaChrom D7000 (Merck-Hitachi, Japan) chromatograph equipped with Perkin Elmer Brownlee Analytical C18 (150 mm, 4.6 mm, 5 μm) column was used. A mobile phase was a mixture of methanol and water (10:90, volume ratio). The analyses were performed under the following conditions: mobile phase flow rate of 0.1 ml/min, injection volume of 20 ml, and detection at λ = 285 nm. Glucose, fructose and sucrose contents were determined by high performance liquid chromatography with refractive index detection (HPLC-RI). The LaChrom D7000 (Merck-Hitachi, Japan) chromatograph equipped with Purospher NH2 (250 nm, 4 mm, 5 mm) column was used. A mobile phase was a mixture of acetonitrile and water (80:20, volume ratio). The analyses were performed under the following conditions: mobile phase flow rate of 0.1 ml/min, injection volume of 20 ml, and temperature of 30ºC. All analyses were performed in triplicate, according to the Regulation of the Minister of Agriculture and Rural Development on methods for evaluation of honey (2009).

Potassium, calcium, magnesium, iron, zinc and manganese contents were determined by atomic absorption spectrometry with flame atomization (F-AAS) (AA240FS, Varian, Australia) according to PN-EN 15505:2009 and PN-EN 14084:2004 Polish Standards, respectively for K, Ca, Mg and for Fe, Zn, Mn. Sample wet digestion was carried out using high pressure microwave oven (Mars Xpress, CEM Corporation, USA), where 10 ml of nitric acid (Suprapur, Merck, Germany) was added to 0.5 g of sample. The process was conducted in a Teflon container at a maximum temperature of 200°C and digestion time of 40 minutes. Mineralized material was diluted with ultra-pure water. The analyses were performed at the following wavelengths: K – 766.5 nm, Ca – 222.7 nm, Mg – 285.2 nm, Fe – 248.3 nm, Zn – 213.9 nm, Mn – 279.5 nm. The recovery of method has been validated using certified reference material (NCS ZC73012 - GSB-5, CNAC, Beijing, China). The methods used were validated and subjected to internal quality control procedure in accordance with PN-EN 13804:2013-6 Polish Standard. Analyses were performed in triplicate.

RESULTS

The lowest water content was found in natural multiflower honeys and it ranged from 19.08 to 20.59 g/100 g, with mean value of 19.84 g/100 g (Table 1). Supplementation of honey with bee products not significantly influenced water content. The highest mean value of water content (20.73 ± 0.93 g/100 g) was observed for the honey supplemented with pollen.

The analyzed honeys were characterized by various insoluble solids content (Table 1), with values of coefficient of variation in the range of 46 – 62%. The lowest mean value of that parameter was found for natural multiflower honeys. Since the bee products are low soluble in water, the supplementation of honey with them significantly affected an increase in insoluble solids content.

Results of determination of the free acids in honeys are presented in Table 1. The highest mean free acids content was found for group of honeys supplemented with beebread (67 meq/kg), while the other groups contained much less free acids, with more than five times lower content of free acids and mean value of 13.2 meq/kg determined in natural multiflower honeys. The remaining samples were characterized by the presence of free acids in the range from 8.5, for a sample of honey with royal jelly, to 43.8 meq/kg, for honey with pollen.

The conductivity (Table 1) of the investigated multifloral honey ranged from 0.18 to 0.40 mS/cm. Supplementation of honey with royal jelly or propolis did not significantly influence this feature. However, in the case of addition of pollen or beebread the increase in conductivity values was observed (Table 1).

In Table 2 the 5-(hydroxymethyl)furfural HMF and sugars content of natural and supplemented with bee products honeys were specified. The HMF content in the tested

Table 1: Quality parameters of natural honeys and honeys supplemented with bee products

Group of samples	Water content (g/100g)	Insoluble solids content (g/100g)	Free acidity (meq/kg)	Conductivity (mS/cm)
Multiflower honeys (n=5)				
Range	19.08–20.59	0.08–0.23	10.50–18.67	0.18–0.40
Mean±SD	19.84±0.69	0.15±0.07	13.20±3.27	0.31±0.10
RSD	3.48	50.70	24.78	30.87
Honeys with royal jelly (n=5)				
Range	19.71–20.45	0.10 – 0.38	8.50–26.17	0.22–0.44
Mean±SD	20.00±0.29	0.26±0.13	15.47±6.87	0.30±0.09
RSD	1.44	50.12	44.45	29.66
Honeys with pollen (n=4)				
Range	19.40–21.10	0.17–0.67	29.83–44.83	0.36–0.50
Mean±SD	20.73±0.93	0.46±0.21	38.00±6.47	0.45±0.07
RSD	3.50	45.53	17.04	14.40
Honeys with beebread (n=3)				
Range	19.63–20.73	0.17–0.83	41.50–87.83	0.54–0.86
Mean±SD	20.21±0.55	0.58±0.36	66.61±23.41	0.72±0.17
RSD	2.73	61.71	35.14	23.11
Honeys with propolis (n=5)				
Range	19.29–20.64	0.17–0.67	12.50–21.50	0.19–0.45
Mean±SD	20.19±0.56	0.37±0.20	18.20±4.11	0.31±0.11
RSD	2.78	53.99	22.57	35.06

Table 2: HMF and sugars content of natural honeys and honeys supplemented with bee products

Group of samples	HMF content (mg/kg)	Fructose (F) content (g/100g)	Glucose (G) content (g/100g)	G+F content (g/100g)	Sucrose content (g/100g)
Multiflower honeys (n=5)					
Range	8.55–13.49	37.11–45.24	27.33–37.76	65.20–74.87	0.57–4.92
Mean±SD	11.06±2.17	41.83±3.29	31.37±3.92	69.74±4.67	2.91±1.99
RSD	19.65	7.87	12.48	6.69	68.30
Honeys with royal jelly (n=5)					
Range	11.08–21.81	35.57–47.81	27.93–38.94	69.57–76.62	0.52–4.74
Mean±SD	14.80±4.27	43.48±4.79	31.41±4.38	72.95±3.26	2.69±1.74
RSD	28.86	11.01	13.93	4.46	64.76
Honeys with pollen (n=4)					
Range	6.67–24.10	35.16–49.78	25.88–30.67	72.58–75.66	1.44–5.65
Mean±SD	12.43±8.16	41.84±6.65	27.52±2.18	74.21±1.27	3.90±1.83
RSD	65.68	15.88	7.91	1.71	46.78
Honeys with beebread (n=3)					
Range	8.69–14.64	34.88–38.40	22.94–26.80	57.82–73.72	2.87–5.58
Mean±SD	12.11±3.07	36.55±1.77	24.53±2.02	66.37±7.09	4.39±1.38
RSD	25.38	4.83	8.22	10.68	31.47
Honey with propolis (n=5)					
Range	9.13–15.51	35.47–47.08	26.32–32.14	60.20–76.57	1.11–5.65
Mean±SD	12.97±2.62	42.20±5.52	28.95±2.55	71.93±7.83	3.90±1.83
RSD	20.18	13.09	8.79	10.89	46.78

samples was from 6.67 to 24.10 mg/kg. The natural multiflower honeys contained 11.06 mg HMF/kg on average. The supplementation of honey with particular bee products did not significantly influence HMF content. The highest average HMF content of 14.8 mg/kg was determined for honeys supplemented with royal jelly, however, the highest HMF content was found in one sample of honeys supplemented with pollen. In this group of samples the greatest variability of results (65%) was also found (Table 2).

The most numerous group of compounds in honey are monosaccharides, including mainly fructose and glucose (Manzanares et al., 2011; 2014). In our study it was found that the highest fructose content of 43.48 g/100 g, expressed as mean value, was in honeys supplemented with royal jelly, while the lowest one (36.55 g/100 g) was in honeys with beebread (Table 2). The highest mean glucose content of 34.41 g/100 g was found in the honeys with royal jelly and in the natural multiflower honeys (31.37 g/100 g). Honeys with beebread were characterized by the lowest glucose content, with mean value of 24.53 g/100 g. The highest average sucrose content (4.39 g/100 g) was determined in group of honeys supplemented with beebread, while the lowest one in the honeys with royal jelly (Table 2).

The natural multiflower honeys tested contained 412 mg of potassium per kg of sample on average, however, potassium content was characterized by a relatively high variability between the samples (Table 3). Supplementation of honeys with royal jelly or propolis did not significantly affect the average potassium content. Significant increase in potassium content was observed in honeys supplemented with pollen or beebread (Table 3). Similar effect was observed for calcium and magnesium. The natural multiflower honeys contained 252 mg/kg of calcium and 29 mg/kg of magnesium, on average (Table 3). Significant increases in calcium and magnesium contents were found for honey with added pollen or beebread. The natural multiflower honeys contained 8.8 mg of iron, 4.5 mg of zinc and 1.4 mg of manganese per kg of sample, on average (Table 3). Supplementation of honeys with royal jelly or propolis did not significantly affect the average content of microelements, contrary to the honeys supplemented with pollen or beebread (Table 3).

DISCUSSION

Water in honey comes from nectar or honeydew and its amount is determined by many factors, which include: climatic conditions, the degree of maturity of honey, geographical origin (honeys from countries with template climate contain more water), humidity and temperature during receiving, conditioning and storage of honey (Anjos et al., 2015). Too high water content of honey has a negative impact on its surface tension, viscosity, consistency, adhesion and cohesion (Witczak et al., 2011). Moreover, high water content of honey creates favorable conditions for a fermentation of honey as a result of the activity of osmophilic yeast. The mean water content of the multiflower honeys is slightly lower than the allowed value (20 g/100 g) given in the Regulation of the Minister of Agriculture and Rural Development. Higher water content in Malaysian and Mexican honeys was reported by A-Rahaman et al. (2013) and Viuda-Martos et al. (2010), respectively. Supplementation of honey with bee products not significantly influenced water content; however, in

Table 3: Mineral composition of natural honeys and honeys supplemented with bee products

Group of samples	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)
Multiflower honeys (n=5)						
Range	204.80–628.34	206.74–315.28	16.81–41.28	4.40–10.71	2.35–6.06	1.14–1.96
Mean±SD	411.99±187.60	252.37±43.04	28.55±10.78	8.78±2.53	4.54±1.60	1.43±0.31
RSD	45.54	17.05	37.77	28.82	35.32	21.83
Honeys with royal jelly (n=5)						
Range	91.93–673.42	176.63–271.09	15.52–44.40	2.92–13.92	1.00–7.43	0.86–2.11
Mean±SD	383.03±222.03	234.58±38.55	27.50±10.49	8.07±4.02	3.60±2.46	1.26±0.52
RSD	57.97	16.43	38.14	49.83	68.24	41.17
Honeys with pollen (n=4)						
Range	494.06±651.22	222.09–406.06	50.99–110.35	5.04–30.50	5.13–20.54	1.64–5.75
Mean±SD	568.49±71.96	328.10±82.85	77.83±26.52	18.07±10.47	12.01±6.91	3.61±1.69
RSD	12.66	25.25	34.08	57.93	57.57	46.86
Honeys with beebread (n=3)						
Range	1100.36–1205.30	382.14–397.30	104.02–195.51	17.95–51.23	10.17–16.71	4.12±7.49
Mean±SD	1145.15±54.13	388.90±7.71	159.24±48.60	33.90±16.68	12.54±3.62	6.32±1.91
RSD	4.73	1.98	30.52	49.21	28.82	30.20
Honey with propolis (n=5)						
Range	102.70–682.18	190.14–289.40	17.73–36.12	1.69–12.50	2.00–7.83	0.59–2.73
Mean±SD	399.29±245.98	238.97±38.56	26.09±6.85	8.17±4.50	4.29±2.28	1.46±0.84
RSD	61.60	16.18	26.24	55.12	53.22	57.43

the case of supplementation with pollen, beebread and propolis the resulting water content expressed as mean value slightly exceeded the maximum allowed value for honey (20 g/100 g). The broadest range of values and the highest mean value of water content were found for honeys supplemented with pollen.

In the composition of honeys the substances insoluble in water are present in small quantities. These include: pollen grains, fragments of bees and other insects, mites, bacterial and fungal spores, fungi and algae cells, residual wax and beebread, mineral crystals, and propolis and royal jelly. Moreover, honey contains trace amounts of lipids. In accordance with the applicable regulations the insoluble solids content of honey should not exceed value of 0.1 g/100 g, with the exception of pressed honey, where the amount could be of 0.5 g/100 g. The bee products are low soluble in water, therefore, the supplementation of honey with them significantly affected an increase in insoluble solids content. In the case of royal jelly, pollen and propolis, the mean insoluble solids content did not exceed value of 0.5 g/100 g. Honeys supplemented with beebread were characterized by the widest range of amounts of water-insoluble substances and by the greatest mean value of that parameter. In this case the greatest variability in that characteristic was found (Table 1), that could result from large variation in the amount of added beebread amounting from 10 to 60%, as declared by the producers. The other groups of honeys, i.e. honeys with royal jelly, pollen and propolis were characterized by much smaller variation in honey supplementation: 0.5-0.8%, 5-10%, and 0.5-1%, respectively. Due to a lack of appropriate standards there is a considerable discretion on the supplementation of honey with bee products.

The free acids content in the multifloral honeys varied in the range of 10.50 – 18.67 meq/kg (Table 1). Much wider range of values of free acids content and higher free acidity were reported by Rodriguez et al. (2012) for Mexican honeys. In accordance with applicable regulations the free acids content should not exceed value of 50 meq/kg. Only two of the tested honey samples were characterized by higher free acids content than the allowable one and they were supplemented with beebread. The remaining samples were characterized by the presence of free acids in the range from 8.5, for a sample of honey with royal jelly, to 43.8 meq/kg, for honey with pollen and these values were consistent with the applicable requirements. Strongly increased free acids content in the honeys supplemented with beebread is due to the fact that the beebread is a mixture of pollen, honey and bee secretions of the salivary glands, which under anaerobic conditions is subjected to fermentation under the influence of bee enzymes and bacteria of the *Lactobacillus* genus. In this process lactic acid is produced among others ones, which lowers the pH and increases acidity of beebread.

The conductivity of honey is determined mainly by the presence of minerals, which in aqueous solution form ions responsible for conduction of electrical current. Moreover, this property depends on the kind of bees benefit used to production of the honey. According to the applicable regulations the conductivity of nectar honeys should be not more than 0.8 mS/cm. Results of our study revealed that only one of the tested samples – honey supplemented with beebread – was characterized by higher conductivity than the allowed value. Natural multiflower honeys showed relatively low values of conductivity, with the mean value of 0.31 mS/cm (Table 1). Similar values of conductivity of the natural honeys were reported by Zhou et al. (2013). Slightly lower conductivity was determined in multiflower honeys by Viuda-Martos et al. (2010). In contrast, much higher conductivity of Malaysian honeys is given by A-Rahaman et al. (2013). Supplementation of honeys with royal jelly or propolis very slightly affected the conductivity of honeys. Although these products are rich sources of nutrients such as amino acids and minerals, which affect the electrical conductivity, relatively low level of honey supplementation (below 1%) resulted in the lack of a significant effect on that parameter. On the contrary, presence of pollen or beebread in the honeys resulted in significant increase in conductivity of the samples due to a much higher honey supplementation with these products as compared to the other ones. Both pollen and beebread are good sources of substances, including organic acids, responsible for the conductivity of honey. High positive correlation between the free acids content and the conductivity of honey ($r = 0.9367$) was found.

The HMF is a compound that is naturally present in honey and is formed by conversion of hexoses in acidic environment. Since fresh honey contains small amounts of HMF, a higher concentration of this compound indicates long term storage of honey, especially at elevated temperatures. Increased HMF content may also indicate overheating of honey during packaging or its adulteration with invert sugar. Nevertheless, the content of this compound in honey is determined by many factors, among which the type of bees benefit, time of collection, climatic conditions and way of storage of honey should be mentioned (Manzanares et al., 2011). According to the applicable regulations, the HMF content in honey should not exceed value of 40 mg/kg, with exception of honeys from tropical regions and their mixtures for which the HMF content should not exceed value of 80 mg/kg. The HMF content in the tested samples (Table 2) indicates that all the tested honeys met the quality requirements. The lowest average content of HMF was observed for natural multiflower honeys. Similar amount of that compound was determined by Manzanares et al. (2011), while significantly lower HMF content in natural honeys was reported by Zhou et al. (2013). In our study, the amounts of HMF in the honeys supplemented with individual bee products were not significant as compared to the natural honeys.

Fructose and glucose contents in nectar honeys usually range between 64 and 89 g/100g, depending on the honey origin (Manzanares et al. 2011, 2014). The highest mean fructose content was determined for honeys supplemented with royal jelly, while the lowest one for honeys with beebread addition (Table 2). The latter phenomenon could be due to the highest level of supplementation of honey with beebread of all the samples. Other groups of honeys showed fructose contents very similar to each other. Honeys with beebread were also characterized by the lowest glucose content of all the samples. According to the applicable regulations, the sum of glucose and fructose content should be not less than 60 g/100 g in nectar honeys. On the basis of our results it can be stated that the largest amounts of these two saccharides were in the honeys supplemented with pollen, with the mean value of 74.21 g/100 g, while the smallest ones were in the honeys with beebread (mean value of 66.37 g/100 g). Again, the reason for the latter phenomenon can be the highest dose of supplementation of honey with beebread as compared to the other bee products. Noteworthy is, however, that the only sample which did not meet the applicable requirements on the sum of fructose and glucose was the honey with added beebread. Sucrose content is one of the quality parameters of honey (Manzanares et al. 2014). Increased sucrose concentration in honey may indicate its adulteration or immaturity. The highest average sucrose content was determined in group of honeys supplemented with beebread, while the lowest one in the honeys with royal jelly (Table 2). According to the applicable regulations sucrose content in honeys should not be higher than 5 g/100 g, and therefore most of the tested samples met this requirement. Only three samples (with beebread, propolis and pollen) obtained from the same apiary were characterized by slightly higher sucrose content (5.58-5.66 g/100 g) than the allowed value. Much higher sucrose contents are reported by Nikolova et al. (2013) for Bulgarian honeys.

Honeys of different origin are characterized by various mineral compositions. Honey is a good source of some of elements, especially of potassium (Bağci et al, 2009; Özcan et al., 2012). In comparison with our results (Table 3) slightly lower potassium content was reported by Chakir et al., (2011) for Moroccan multiflower honeys, while significantly higher one were given by Zhou et al. (2013) for Chinese honeys. Supplementation of honeys with royal jelly or propolis did not significantly affect the average potassium content, that was due to small addition (below 1%) of these products to the honeys. Significant increase in potassium content was observed in honeys supplemented with pollen or beebread (Table 3). In the latter case the average potassium content increased almost threefold. Both pollen and beebread are good sources of mineral elements (Orzáez Villanueva et al. 2001). Therefore, as it should be assumed, the highest potassium content determined in the honeys with beebread came from the highest level of supplementation of honey with this product. Similar effect was observed for calcium and magnesium. In comparison with our results significant lower amounts of calcium and magnesium in natural Chinese, Moroccan and Turkish honeys were determined by Zhou et al. (2013), Chakir et al. (2011) and Bağci et al. (2009). The average contents of these elements in honeys supplemented with royal jelly or propolis were at very similar level. Significant increases in calcium and magnesium contents were found for honey with added pollen or beebread (Table 3), with greater effect observed for potassium. Significantly greater increase in mineral element content was stated for magnesium. Honeys with beebread were characterized by more than five times higher magnesium content than the natural multiflower honeys. The microelement contents determined in the multiflower honeys (Table 3) were at the levels similar to these reported in the literature (Chakir et al. 2011, Pohl and Sergiel 2010, Stecka et al., 2014). Slightly higher iron and zinc contents in multiflower honeys were found by Perna et al., (2011). Similarly to the case of macroelements, supplementation of honeys with royal jelly or propolis did not significantly affect the average microelement contents (Table 3), contrary to the supplementation of honeys with pollen or beebread. The highest average contents of iron, zinc and manganese were determined in honeys supplemented with beebread. The individual microelement contents were from three to four times greater than in the natural multiflower honeys. As mentioned above, this dependence was due to the highest level of supplementation for beebread of all the samples.

CONCLUSIONS

On the basis of the obtained results it was found that supplementation of honeys with bee products slightly influenced their water and HMF contents. Addition of beebread to the natural honeys resulted in decreased sum of glucose and fructose, due to the highest level of supplementation of honey with this product. Honeys supplemented with pollen and beebread were characterized by slightly greater sucrose content than natural honeys. Addition of the individual bee products significantly affected an increase in insoluble solids content, free acids content and conductivity of the honeys that resulted from the nature of the bee products used. The greatest effect was found for honeys supplemented with pollen or beebread, since these products were added to the honeys at the highest amounts. Presence of pollen or beebread in honey significantly affected potassium, calcium, magnesium, iron, zinc and manganese contents. Introduction of bee products into the human diet through the supplementation of honey seems to be the most appropriate method; however, it may adversely affect certain honey quality parameters. Using the bee products for supplementation of natural honey requires further studies on the effect of these additives on sensory attractiveness of the resulting products and on the presence of selected bioactive compounds.

FINANCIAL SUPPORT

This research was financed by the Ministry of Science and Higher Education of the Republic of Poland.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors

Authors' contributions

Lesław Juszczak conceived and designed the experiments, analysed the data and prepared the manuscript; Adam Florkiewicz, Robert Socha and Anna Piotrowska performed the experiments; Dorota Gałkowska analyzed the data and prepared the manuscript.

REFERENCES

Afrin, S., F. Giampieri, T. Y. Forbes-Hernández, M. Gasparrini, A. Amici, D. Cianciosi, J. L. Quiles and M. Battino. 2018. Manuka honey synergistically enhances the chemopreventive effect of 5-fluorouracil on human colon cancer cells by inducing oxidative stress and apoptosis, altering metabolic phenotypes and suppressing metastasis ability. Free Radic. Biol. Med. 126: 41-54.

Anjos, O., C. Iglesias, F. Peres, J. Martinez, A. Garcia and J. Taboada. 2015. Neural networks applied to discriminate botanical origin of honeys. Food Chem. 175: 128-136.

A-Rahaman, N. L., L. S. Chua, M. R. Sarmidi and R. Aziz. 2013. Physicochemical and radical scavenging activities of honey samples from Malaysia. Agricu. Sci. 4(5B): 46-51.

Bağci, Y., D. Arslan, M. M. Őzcan and N. Dursun. 2007. Determination of the mineral content of bee honeys produced in Middle Anatolia. Int. J. Food Sci. Nutr. 58: 567-575.

Chakir, A., A. Romane, N. Barbagianni, D. Bartoli and P. Ferrazzi. 2011. Major and trace elements in different types of Moroccan honeys. Austral. J. Basic Appl. Sci. 5(4): 223-231.

Farooqui, T and Farooqui, A. A. (2012). Beneficial effect of propolis on human health and neurological diseases. Front. Biosci. E4: 779-793.

Gasparrini, M., S. Afrin, T. Y. Forbes-Hernández, D. Cianciosi, P. Reboredo-Rodriguez, A. Amici, M. Battino and F. Giampieri. 2018. Protective effects of Manuka honey on LPS-treated Raw 264.7 macrophages. Part 2: Control of oxidative stress induced damage, increase of antioxidant enzyme activities and attenuation of inflammation. Food Chem. Toxicol. 120: 578-587.

Habib, H. M., F. T. Al Meqbali, H. Kamal, U. D. Souka and W. H. Ibrahim. 2014. Bioactive components, antioxidant and DNA damage inhibitory activities of honeys from arid regions. Food Chem. 153: 28-34.

Hongcheng, Z., W. Xue, W. Kai and L. Chunyang. 2015. Antioxidant and tyrosinase inhibitory properties of aqueous ethanol extracts from monofloral bee pollen. J. Apicul. Sci. 59(1): 119-128.

Isidorov, V. A., A. G. Isidorova, L. Szczepaniak and U. Czyżewska. 2009. Gas chromatographic – Mass spectrometric investigation of the chemical composition of beebread. Food Chem. 115: 1056-1063.

Jamróz, M. K., K. Paradowska, K. Zawada, K. Makarova, S. Kaźmierski and I. Wawer. 2014. ^{1}H and ^{13}C NMR-based sugar profiling with chemometric analysis and antioxidant activity of herbhoneys and honeys. J. Sci. Food Agric. 94: 246-255.

Juszczak, L., D. Gałkowska, M. Ostrowska and R. Socha. 2016. Antioxidant activity of honey supplemented with bee products. Nat. Prod. Res. 30(12): 1436-1439.

Llnskens, H. F and W. Jorde. 1997. Pollen as food and medicine - A review. Econ. Bot. 51(1): 78-86.

Manzanares, A. B., Z. H. García, B. R. Galdón and E. R. Rodríguez. 2011. Differentiation of blossom and honeydew honeys using multivariate analysis on the physicochemical parameters and sugar composition. Food Chem. 126(2): 664-672.

Manzanares, A.B.,Z. H. García, B. R. Galdón, E. R. Rodríguez and C. D. Romero. 2014. Physicochemical characteristics of minor monofloral honeys from Tenerife, Spain. LWT-Food Sci. Technol. 55(2): 572-578.

Nikolova, K., G. Gentscheva and E. Ivanova. 2013. Survey of the mineral content and some physicochemical parameters of Bulgarian bee honeys. Bulgarian Chem. Commun. 45(2): 244-249.

Özcan, M. M., C. BÖlmez, D. Arslan and N. Dursun. 2012. Mineral and heavy metal contents of different honeys produced in Turkey. J. Apicul. Res. 51(4): 353-358.

Perna, A., A. Simonetti, I. Intaglietta, A. Sofo and E. Gambacorta. 2012. Metal content of southern Italy honey of different botanical origins and its correlation with polyphenol content and antioxidant activity. Int. J. Food Sci. Technol. 47(9): 1909-1917.

Pohl, P and I. Sergiel. 2010. Direct determination of the total concentrations of copper, iron and manganese and their fractionation forms in freshly ripened honeys by means of flame atomic absorption spectrometry. Microchimca Acta. 168: 9-15.

Polish Standard PN-EN 15505:2009. Foodstuffs. Determination of Trace Elements. Determination of Sodium and Magnesium by Flame Atomic Absorption Spectrometry (AAS) After Microwave Digestion.

Polish Standard PN-EN 14084:2004. Foodstuffs. Determination of Trace Elements. Determination of Lead, Cadmium, Zinc, Copper and Iron by Atomic Absorption Spectrometry (AAS) After Microwave Digestion.

Polish Standard PN-EN 13084:2013-06. Foodstuffs. Determination of Elements and their Chemical Species. General Considerations and Specific Requirements.

Rady, H. M., K. Z. Hemmaid, N. N. Esmaeil, M. M. Eid and A. A. Elshat. 2018. Sidr Kashmiry honey and its fractions induced apoptosis in hepatocellular carcinoma *in vitro*. Mediterr. J. Nutr. Metabol. 11(3): 343-351.

Ramadan, M. F and A. Al-Ghamdi. 2012. Bioactive compounds and health-promoting properties of royal jelly: A review. J. Funct. Foods. 4(1): 39-52.

Regulation of the Minister of Agriculture and Rural Development on the Detailed Requirements of the Commercial Quality of Honey. 2003. ???. ??? . 181: 1773.

Regulation of the Minister of Agriculture and Rural Development on Methods for Evaluation of Honey. 2009. ???. ??? . 17: 94.

Rodriguez, B. A., S. Mendoza, M. H. Iturriga and E. Castaño-Tostado. 2012. Quality parameters and antioxidant and antibacterial properties of some Mexican honeys. J. Food Sci. 71(1): 121-127.

Sahinler, N and O. Kaftanoglu. 2005. Natural product propolis: Chemical composition. Nat. Prod. Res. 19(2): 183-186.

Socha, R., D. Gałkowska, M. Bugaj and L. Juszczak. 2015. Phenolic composition and antioxidant activity of propolis from various regions of Poland. Nat. Prod. Res. 29(5): 416-422.

Socha, R., C. Habryka and L. Juszczak. 2016. The effect of enrichment of honey with propolis on the phenolic compounds content and antioxidant activity. Food. Sci. Technol. Qual. 5(108): 127-139.

Socha, R., C. Habryka and L. Juszczak. 2018. Effect of bee bread additive on content of phenolic compounds and antioxidant activity of honey. Food. Sci. Technol. Qual. 2(115): 108-119.

Stecka, H., D. Jedryczko, M. Wełna and P. Pohl. 2014. Determination of traces of copper and zinc in honeys by the solid phase extraction pre-concentration followed by the flame atomic absorption spectrometry detection. Environ. Monit. Assess. 186(10): 6145-6155.

Villanueva, M. T. O., A. D. Marquina, R. B. Serrano and G. B. Abellána. 2001. Mineral content of commercial pollen. Int. J. Food Sci. Nutr. 52(3): 243-249.

Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernández-López and J. A. Pérez-Álvarez. 2008. Functional properties of honey, propolis and royal jelly. J. Food Sci. 73(9): 117-124.

Viuda-Martos, M., Y. Ruiz-Navajas, J. M. Zaldivar-Cruz, V. Kuri, Fernández-López,, Carbonell-Barrachina, Á.A., and Pérez-Álvarez, J.Á. 2010. Aroma profile and physico-chemical properties of artisanal honey from Tabasco, Mexico. International Journal of Food Science and Technology 45,6: 1111-1118.

Witczak, M., L. Juszczak and D. Gałkowska. 2011. Non-newtonian behaviour of heather honey. J. Food Eng. 104(4): 532-537.

Zhou, J., Z. Suo, P. Zhao, N. Cheng, H. Gao, J. Zhao and W. Cao. 2013. Jujube honey from China: Physicochemical characteristics and mineral contents. J. Food Sci. 78(3): 387-394.