doi: 10.17221/350/2016-CJFS

Antimicrobial and Antioxidant Properties of Some Commercial Honeys Available on the Polish Market

Małgorzata MAKAREWICZ[1]*, Stanisław KOWALSKI[2], Marcin LUKASIEWICZ[2] and Magdalena MAŁYSA-PAŚKO[2]

[1]Department of Fermentation Technology and Technical Microbiology and [2]Department of Carbohydrate Technology, Faculty of Food Technology, University of Agriculture in Krakow, Krakow, Poland

*Corresponding author: rrmakare@cyf-kr.edu.pl

Abstract

Makarewicz M., Kowalski S., Lukasiewicz M., Małysa-Paśko M. (2017): Antimicrobial and antioxidant properties of some commercial honeys available on the Polish market. Czech J. Food Sci., 35: 401–406.

Six commercial natural honeys available on the Polish market were characterised with respect to their geographical and floral origins, physicochemical parameters and microbial properties. The study focused on a determination of the activity of the main enzymes, antioxidant capacity and identification of antimicrobial effects. Fructose was the predominant sugar in all tested honeys. The largest amount of hydroxymethylfurfural (HMF) was found in eucalyptus honey. It was found that thyme honey was characterised by the highest values of diastase number, invertase activity, antioxidant capacity and total phenolic content. A very week correlation between the antioxidant properties of tested honeys and their antimicrobial action against tested bacteria was observed. *M. luteus* and *P. putida* were resistant to most honey samples. All tested honeys showed antibacterial activity against *E. coli* and *P. myxofaciens*. *B. subtilis* was resistant only to eucalyptus honey.

Keywords: bee product; enzymatic activity; HMF; microbial properties; antioxidants; phenolic content

Honey produced by *Apis mellifera* is not only one of the oldest sweeteners, but is also a traditional medicine employed in the treatment of several human ailments (Elbanna *et al.* 2014).

It is known that this natural product is effective in the treatment of heart disease, cancer and several inflammatory diseases. Many studies have indicated that its antioxidant activity results in a reduced level of oxidative reactions within food systems and also in the human body. First and foremost, honey is known to be rich in sugars, but compounds such as minerals, vitamins, enzymes, ascorbic acid, carotenoid derivatives, flavonoids, organic acids, Maillard reaction products, free amino acids and other phytochemicals are also present (Liu *et al.* 2013; Elbanna *et al.* 2014; Can *et al*. 2015). Many reports have demonstrated the antibacterial activity of honey and have found that natural honey has broad-spectrum antibacterial activity against pathogenic bacteria and food spoilage microorganisms, including aerobes and anaerobes, Gram-positive (G^+) and Gram-negative (G^-) bacteria (Mandal & Mandal 2011; Liu *et al.* 2013). Increasing globalisation means that products of different geographical origin can easily be purchased at local stores. Therefore, it is of interest to compare the properties of various natural honeys.

The present study aimed to characterise five commercial honeys of different geographical and floral origins available on the Polish market.

Supported by theMinistry of Science and Higher Education, Republic of Poland, Grants No. DS3706/KTFiMT/2016 and No. DS3709/KTW/2016.

doi: 10.17221/350/2016-CJFS

MATERIAL AND METHODS

Honey samples characterisation. Sunflower (*Helianthus*) and coriander (*Coriandrum*) honeys from the Black Sea region; eucalyptus (*Eucalyptus*) from the Amazon basin; thyme (*Thymus vulgaris*) and lavender (*Lavendula*) from the Mediterranean region; and lime (*Tilia*) honey from Poland were purchased from beekeeping cooperatives (Bartnik Sadecki, Poland). The authenticity of all honey samples was verified. Basic physicochemical properties of honey such as electrical conductivity, moisture content, acidity, hydroxymethylfurfural (HMF) content, carbohydrate profile, diastase, and invertase activity were carried out according to methods of the International Honey Commission (Bogdanov 2002).

Glucose oxidase (GO) activity was determined based on the H_2O_2 detection method developed by Mottola *et al*. (1970) and modified by Lukasiewicz *et al.* (2015). Total phenolic content was measured using the Folin-Ciocalteu reagent (Meda *et al.* 2005). The antioxidant activity of honeys was determined according to a method described in our previous publication (Lukasiewicz *et al.* 2015). The method is based on the work of Baltrusaityte *et al.* (2007), and earlier studies by Re *et al.* (1999) and Turkmen *et al*. (2006). All measurements were done in duplicate.

Antimicrobial activity measurement. Five different microorganisms were used: *Escherichia coli* (DSMZ 20030), *Micrococcus luteus* (DSMZ 4261), *Proteus myxofaciens* (DSMZ 4482), *Bacillus subtilis* (DSMZ 10), and *Pseudomonas putida* (DSMZ 291). Microbial strains were purchased from the German Collection of Microorganisms and Cell Cultures (Leibniz Institute, DSMZ). Bacterial in all samples were enumerated using the McFarland densitometer method (Silici *et al*. 2010). Well diffusion assays were performed to evaluate the antibacterial activity of each sample as described previously (Lukasiewicz *et al.* 2015).

Statistical analysis. Analysis was carried out using Statistica 9.0 (StatSoft, Poland). Analysis of variance and determination of the statistical significance of differences were performed using Tukey's and Duncan's tests at $P < 0.05$.

RESULTS AND DISCUSSION

Water content in the analysed honeys ranged from 16.5% (lime) to nearly 18.0% (eucalyptus). These values indicate the appropriate maturity because water content should not exceed 20% (Council Directive 2001). The sum of glucose and fructose was above 60 g/100 g in all tested honeys, which is the value required for this type of honey; the predominant sugar in all tested samples was fructose (Table 1). Sucrose was not detected in any of the analysed honeys. This fact is somewhat surprising since in eucalyptus and lavender honey sucrose contents of 10 and 15%, respectively, are allowed. On the other hand, it is known that with storage time, the sucrose content in honey decreases due to invertase activity, which catalyses the hydrolysis of sucrose to an equimolar mixture of monosaccharides (fructose and glucose). Sucrose content can fall by as much as 80% within six months (Rybak-Chmielewska 2007).

Among the tested honeys, eucalyptus honey was characterised by the highest specific conductivity (above 1 µS/cm); the specific conductivity of the other honeys did not exceed 0.6 mS/cm. The specific conductivity of floral honey should not exceed 0.8 µS/cm (Council Directive 2001), but eucalyptus honey is an exception; therefore, it must be concluded that the resulting value is permitted for this type of honey. Moreover, this value is within the range reported by other authors (up to 1.141 µS/cm) (Chakir *et al*. 2016), although most sources indicate values not exceeding 0.6 µS/cm (Silva *et al*. 2009; Makhloufi *et al.* 2010).

Acidity did not exceed the permissible value of 50 mM NaOH/kg for any of the tested honeys. Eucalyptus honey was characterised by the highest acidity, and sunflower honey had the lowest acidity value (Table 1). Slight changes in the values of these parameters can be due to the season in which the honey is collected. It was found that honeys from spring and early summer exhibit lower acidity compared to those from later forages (Semkiw *et al.* 2010). Acidity can also increase over time, as a result of fermentation caused by microbiological spoilage (Da Silva *et al.* 2016).

In accordance with the current EU legislation, HMF concentration should not exceed 40 mg/kg, except for honeys from tropical countries in which case values of up to 80 mg/kg are permitted. Among the tested samples, only thyme and lime honeys fulfilled these regulatory requirements; in all other cases, the content of HMF exceeded the value stipulated in the guidelines. The largest amount of this compound (about 100 mg/kg) was found in eucalyptus honey (Table 1). HMF content in honey is affected by several factors such as time and temperature of storage and

doi: 10.17221/350/2016-CJFS

Table 1. Physicochemical parameters of honeys

Honey type	Fructose	Glucose	Maltose	F/G	HMF (mg/kg)	Water (%)	Conductivity (mS/cm)	Acidity		
								free	lactone	total
	(g/100 g)							(mmol NaOH/kg)		
Sunflower	$47.18^{a} \pm 0.78$	$37.52^{a} \pm 2.48$	$1.92^{b} \pm 0.11$	1.26	$58.53^{c} \pm 1.83$	$17.3^{b} \pm 0.2$	$0.357^{e} \pm 0.002$	$8.27^{d} \pm 0.58$	$8.06^{b} \pm 1.46$	$16.34^{d} \pm 0.88$
Eucalyptus	$41.84^{b} \pm 1.50$	$37.93^{a} \pm 1.42$	$1.88^{b} \pm 0.11$	1.10	$100.38^{a} \pm 1.20$	$17.9^{a} \pm 0.1$	$1.039^{a} \pm 0.005$	$14.40^{a} \pm 0.58$	$11.11^{a} \pm 0.58$	$25.51^{a} \pm 0.00$
Thyme	$41.83^{b} \pm 1.03$	$26.34^{b} \pm 1.84$	$6.60^{a} \pm 1.77$	1.59	$18.81^{e} \pm 0.71$	$17.7^{ab} \pm 0.2$	$0.505^{b} \pm 0.001$	$13.79^{ab} \pm 0.87$	$8.85^{b} \pm 0.29$	$22.64^{b} \pm 1.16$
Lavender	$46.33^{a} \pm 1.81$	$34.68^{a} \pm 1.22$	$2.47^{b} \pm 0.08$	1.34	$57.94^{c} \pm 1.15$	$17.8^{a} \pm 0.1$	$0.270^{f} \pm 0.001$	$10.48^{c} \pm 0.87$	$8.63^{b} \pm 0.58$	$19.12^{c} \pm 0.29$
Coriander	$43.74^{ab} \pm 1.87$	$33.93^{a} \pm 1.29$	$6.64^{a} \pm 0.15$	1.29	$67.60^{b} \pm 0.19$	$15.9^{d} \pm 0.2$	$0.414^{d} \pm 0.003$	$12.78^{b} \pm 0.30$	$7.12^{b} \pm 0.59$	$19.90^{c} \pm 0.89$
Lime	$41.36^{b} \pm 1.42$	$34.22^{a} \pm 1.31$	$6.43^{a} \pm 0.70$	1.21	$26.41^{d} \pm 0.69$	$16.5^{c} \pm 0.1$	$0.444^{c} \pm 0.001$	$7.09^{d} \pm 0.59$	$8.97^{b} \pm 0.88$	$16.06^{d} \pm 0.29$

Values in columns which do not differ statistically significantly were marked with the same letters. α = 0.05; F/G – fructose to glucose ratio

duration of thermal treatment (Sancho *et al.* 1992; Karabournioti & Zervalaki 2001; Kowalski 2013; Kowalski *et al.* 2013). We therefore suspect that the high levels of this compound in honeys was the result of their storage and packaging and linked to import and packaging into individual containers.

Apart from the above characteristics, amylolytic enzyme activity, so-called diastase number, is also used to assess honey quality. In accordance with European legislation, this value should be above 8°C on the Schade scale. The obtained values of diastase number, especially in the context of the HMF content, may indicate improper production of honey, although there are reports indicating low activity of amylolytic enzymes in eucalyptus honey (about 6°C) (Chakir *et al.* 2016). On the other hand, Makhloufi *et al.* (2010) observed diastase number in eucalyptus honey to range from 8 to 30, HMF content from 6 to 110 mg/kg and invertase activity to range from 1 to 20. In our study, the invertase activity of eucalyptus honey was not considerably lower (0.7). The highest activity of this enzyme was observed in thyme honey (Table 2). However, it should be noted that invertase is a very storage-sensitive enzyme (Bonvehí *et al.* 2000), and its activity can decline significantly over time (Orantes-Bermejo & Torres Fernández-Píñar 2009; Lichtenberg-Kraag 2012).

A strong variation in GO content was observed in all samples, ranging from 4.5 mg of hydrogen peroxide per kg of honey (lime honey) to 73 mg for the coriander honey sample. Such different values may result from the high sensitivity of GO enzymes, which together with long storage and transport times, may result in decreased enzyme content. GO is an important factor with respect to the antimicrobial activity of honey, mainly due to hydrogen peroxide

Table 2. Enzymatic activity of honey samples

Honey type	DN (Schade)	IN (°C)	GO mg H_2O_2/kg/24 h
Sunflower	$5.8^{d} \pm 0.4$	$0.5^{f} \pm 0.0$	11.1 ± 1.1
Eucalyptus	$4.7^{d} \pm 0.1$	$0.7^{e} \pm 0.1$	15.9 ± 4.0
Thyme	$20.9^{a} \pm 1.6$	$6.2^{a} \pm 0.0$	33.2 ± 2.7
Lavender	$7.8^{c} \pm 0.4$	$1.2^{d} \pm 0.0$	36.388
Coriander	$8.2^{c} \pm 0.6$	$3.0^{c} \pm 0.0$	73.634
Lime	$10.4^{b} \pm 0.1$	$4.1^{b} \pm 0.1$	4.5 ± 1.2

DN – Diastase number; IN – Invertase number; GO – Glucose oxidase activity; values in columns which do not differ statistically significantly were marked with the same letters; α = 0.05

doi: 10.17221/350/2016-CJFS

Table 3. Antioxidant properties of honey

Honey type	Antioxidant power			Total phenolic content (μM GA/g)
	(μM DPPH/g)	(μM $ABTS^+$/g)	(μM Fe^{2+}/g)	
Sunflower	$4.84^{f} \pm 0.10$	$54.13^{f} \pm 3.39$	$1.15^{f} \pm 0.03$	$1.36^{e} \pm 0.01$
Eucalyptus	$18.37^{b} \pm 0.16$	$308.89^{b} \pm 4.77$	$4.69^{b} \pm 0.04$	$3.66^{b} \pm 0.05$
Thyme	$43.60^{a} \pm 0.54$	$371.25^{a} \pm 4.15$	$10.81^{a} \pm 0.12$	$5.11^{a} \pm 0.00$
Lavender	$5.76^{e} \pm 0.24$	$104.97^{d} \pm 11.46$	$1.60^{e} \pm 0.02$	$1.96^{d} \pm 0.05$
Coriander	$10.51^{c} \pm 0.12$	$136.21^{c} \pm 0.83$	$3.87^{c} \pm 0.02$	$2.88^{c} \pm 0.02$
Lime	$7.36^{d} \pm 0.04$	$85.53^{e} \pm 0.20$	$2.17^{d} \pm 0.01$	$1.91^{d} \pm 0.03$

Values in columns which do not differ statistically significantly were marked with the same letters; α = 0.05

that is produced as a side reaction product. The specific composition of essential oils in plants such as thyme, coriander and lavender are known to confer antiseptic properties (Peter 2012). Honey obtained from those plants were observed to possess high oxidase activity, which may suggest some connection between the action of essential oils and the stability of oxidase in the product.

Regardless of the method of antioxidant activity determination, the highest antioxidant capacity was found in thyme honey; in contrast, sunflower honey exhibited the lowest ability to quench of free radicals (Table 3). The antioxidant capacities of tested honeys was in descending order: thyme > eucalyptus > coriander > lavender > lime > sunflower honey. Total phenolic content exhibited a similar trend with the exception that lavender and lime honeys did not differ significantly. The above observations are to some extent in line with those of Alves *et al.* (2013) regarding Portuguese honeys. They reported that thyme honey has a higher ability to scavenge free DPPH radicals than eucalyptus honey. On the other hand, these authors also demonstrated an increased ability of eucalyptus honey to reduce iron ions and a higher content of total polyphenols in this honey compared to thyme honey. It seems, however, that such differences may be due to the geographical origin of honey (Al-Mamary *et al.* 2002; Gheldof *et al.* 2002; Can *et al*. 2015). The relatively low total polyphenol content of lavender honey as well as its low ability to reduce iron ions (FRAP) was confirmed also by Alzahrani *et al*. (2012).

The tested honeys exhibited varying degrees of antibacterial activity against different bacteria as determined by the diameters of the inhibition zones. In the first step of the experiment, the effects of 75% honey extracts were studied on all tested microorganisms (Table 4). The most sensitive bacteria (*E. coli*, *P. myxofaciens*, and *B. subtilis*) were selected for the second part of the analysis (Table 5). The antimicrobial activity of chloramphenicol (positive control) was observed against all tested strains. The results shown in Table 4 demonstrate that not all tested bacteria were sensitive to honeys. *M. luteus* and *P. putida* were resistant to most samples. *B. subtilis* was resistant only to eucalyptus honey. However, all tested honeys exhibited antibacterial activity against *E. coli* and *P. myxofaciens* (Table 4). The antimicrobial activity of phenolic compounds is well known. Some authors maintain that flavonoids and other phenolic components in nectar have antimicrobial activity and inhibit the growth of

Table 4. Antimicrobial activity of 75% honeys solutions (diameter of inhibition zones, mm)

Honey type (75% w/w)	Gram-positive			Gram-negative	
	B. subtilis	*M. luteus*	*E. coli*	*P. myxofaciens*	*P. putida*
Sunflower	$34.3^{a} \pm 0.6$	$10.7b^{c} \pm 0.6$	$31.7^{ab} \pm 1.2$	$31.7^{a} \pm 0.6$	$0.0^{d} \pm 0.0$
Eucalyptus	$0.0^{d} \pm 0.0$	$12.0^{b} \pm 0.0$	$31.3^{ab} \pm 0.6$	$30.0^{b} \pm 1.5$	$0.0^{d} \pm 0.0$
Thyme	$34.3^{a} \pm 0.6$	$0.0^{d} \pm 0.0$	$31.7^{ab} \pm 0.6$	$31.7^{a} \pm 0.6$	$0.0^{d} \pm 0.0$
Lavender	$34.3^{a} \pm 0.6$	$6.7^{c} \pm 5.8$	$32.7^{a} \pm 1.5$	$31.7^{a} \pm 0.6$	$0.0^{d} \pm 0.0$
Coriander	$34.4^{a} \pm 1.5$	$0.0^{d} \pm 0.0$	$31.3^{ab} \pm 0.6$	$28.7^{b} \pm 1.2$	$8.7^{c} \pm 1.2$
Lime	$32.3^{b} \pm 0.6$	$0.0^{d} \pm 0.0$	$31.0^{b} \pm 0.0$	$29.3^{b} \pm 1.2$	$10.3^{b} \pm 1.5$
Chloramphenicol*	$22.2^{c} \pm 0.3$	$32.0^{a} \pm 0.5$	$15.3^{c} \pm 0.3$	$18.7^{c} \pm 0.7$	$14.3^{a} \pm 1.0$

Values in columns which do not differ statistically significantly were marked with the same letters; α = 0.05; *positive control

doi: 10.17221/350/2016-CJFS

Table 5. Antimicrobial activity of honeys (diameter of inhibition zones, mm) for *Escherichia coli*, *Proteus myxofaciens* and *Bacillus subtilis*

Honey (% w/w)	Sunflower			Eucalyptus			Thyme			Lavender			Coriander			Lime		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
5	0.0^{d}	0.0^{c}	0.0^{d}	17.6^{d}±0.6	0.0^{c}	0.0^{a}	0.0^{c}	0.0^{c}	0.0^{c}	15.3^{d}±0.6	0.0^{c}	0.0^{b}	11.7^{c}±0.6	0.0^{b}	0.0^{c}	13.3^{c}±0.6	0.0^{b}	0.0^{a}
10	19.3^{c}±2.1	0.0^{c}	8.7^{c}±1.2	20.7^{c}±1.2	0.0^{c}	0.0^{a}	17.3^{b}±1.2	16.0^{b}±1.7	0.0^{c}	20.3^{c}±1.2	0.0^{c}	0.0^{b}	21.3^{b}±1.2	0.0^{b}	16.0^{b}±1.0	20.0^{b}±2.0	0.0^{b}	0.0^{a}
25	25.0^{b}±1.0	21.0^{b}±1.7	20.0^{b}±0.0	26.0^{b}±0.0	21.7^{b}±1.2	0.0^{a}	23.3^{a}±1.2	22.3^{a}±0.6	11.7^{b}±2.9	23.7^{b}±1.2	20.3^{b}±0.5	0.0^{b}	25.3^{a}±0.6	0.0^{b}	26.3^{a}±0.6	24.3^{a}±3.8	0.0^{b}	0.0^{a}
50	28.3^{a}±0.6	28.7^{a}±2.3	25.3^{a}±1.2	28.7^{a}±1.2	26.3^{a}±1.2	0.0^{a}	25.3^{a}±1.2	25.3^{a}±4.7	21.7^{a}±1.2	27.7^{a}±0.6	26.3^{a}±1.5	15.3^{a}±1.2	26.3^{a}±0.6	26.0^{a}±0.0	26.7^{a}±0.6	26.3^{a}±2.1	23.7^{a}±1.5	0.0^{a}

Values in columns which do not differ statistically significantly were marked with the same letters. α = 0.05; 1 – *Escherichia coli*; 2 – *Proteus myxofaciens*; 3 – *Bacillus subtilis*

a wide range of Gram-negative and Gram-positive bacteria. Suggested mechanisms for the antibacterial activity of polyphenols include membrane disruption, metal ion complexation and enzyme inhibition by polyphenols (Elbanna *et al.* 2014). It should be noted that in this experiment a very weak correlation was observed between the antioxidant properties of tested honeys and their antimicrobial action against the tested microorganisms. We observed a correlation between these parameters only in the case of thyme honey (Tables 2, 4, and 5). In other cases, there were no such direct interconnections; for example, sunflower honey, with the lowest antioxidant capacity (Table 2), demonstrated high antibacterial activity (Tables 4 and 5). Similar results were obtained by Isidorov *et al.* (2015) and by Lukasiewicz *et al.* (2015). These results suggest that the antimicrobial activities of different monofloral honeys are mainly dependent on plant source. This may be related to the fact that most plants contain an extensive number of polyphenols and flavonoids and each plant tends to have its own distinct profile (Silici *et al*. 2010; Isidorov *et al.* 2015). Moreover, several authors have concluded that honeys from certain plants have better antimicrobial activities than those from others (Elbanna *et al*. 2014). Consequently, the relatively high antimicrobial activity of thyme honey against the tested bacteria could be associated with the presence of heat-resistant bioactive compounds with strong antimicrobial activity such as thymol and carvacrol. In addition, the antibacterial properties of honeys are also derived from the antiseptic properties of H_2O_2 (Mandal & Mandal 2011; Elbanna *et al*. 2014), which results from high GO activity. The correlation between GO activity and antimicrobial capacity was also observed for lime honey, which had the lowest GO activity in this study.

References

Al-Mamary M., Al-Meeri A., Al-Habori M. (2002): Antioxidant activities and total phenolics of different types of honey. Nutrition Research, 22: 1041–1047.

Alves A., Ramos A., Gonçalves M. M., Bernardo M., Mendes B. (2013): Antioxidant activity, quality parameters and mineral content of Portuguese monofloral honeys. Journal of Food Composition and Analysis, 30: 130–138.

Alzahrani H. A., Alsabehi R., Boukraâ L., Abdellah F., Bellik Y., Bakhotmah B. A. (2012): Antibacterial and antioxidant potency of floral honeys from different botanical and geographical origins. Molecules, 17: 10540–10549.

doi: 10.17221/350/2016-CJFS

Baltrusaityte V., Venskutonis P. R., Ceksteryte V. (2007): Radical scavenging activity of different floral origin honey and beebread phenolic extracts. Food Chemistry, 101: 502–514.

Bogdanov S. (2002): Harmonised methods of the International Honey Commission. International Honey Commission,

Bonvehi J.S., Torrento M.S., Raich J.M. (2000): Invertase activity in fresh and processed honeys. Journal of the Science of Food and Agriculture, 80: 507–512.

Can Z., Yilidz O., Sahin H., Turumtay E.A., Silici S., Kolayli S. (2015): An investigation of Turkish honeys: their physico-chemical properties, antioxidant capacities and phenolic profiles. Food Chemistry, 180: 133–141.

Chakir A., Romane A., Marcazzan G.L., Ferrazzi P. (2016): Physicochemical properties of some honeys produced from different plants in Morocco. Arabian Journal of Chemistry, 9 (Suppl. 2): S946–S954.

Council Directive (2001): Council Directive of 20 December relating to honey 2001/110/EC. Official Journal of the European Communities, L 010, 12/01/2002: 47–52.

Da Silva P.M., Gauche C., Gonzaga L.V., Costa A.C.O., Fett R. (2016): Honey: chemical composition, stability and authenticity. Food Chemistry, 196: 309–323.

Elbanna K., Attalla K., Elbadry M., Abdeltawab A., Gamal-Eldin H., Fawzy Ramadan M. (2014): Impact of floral sources and processing on the antimicrobial activities of different unifloral honeys. Asian Pacific Journal of Tropical Disease, 4: 194–200.

Gheldof N., Wang X.-H., Engeseth N.J. (2002): Identification and quantification of antioxidant components of honeys from various floral sources. Journal of Agricultural and Food Chemistry, 50: 5870–5877.

Isidorov V.A., Bagan R., Bakier S., Swiecicka I. (2015): Chemical composition and antimicrobial activity of Polish herbhoneys. Food Chemistry, 171: 84–88.

Karabournioti S., Zervalaki P. (2001): The effect of heating on honey HMF and invertase. Apiacta, 36: 177–181.

Kowalski S. (2013): Changes of antioxidant activity and formation of 5-hydroxymethylfurfural in honey during thermal and microwave processing. Food Chemistry, 141: 1378–1382.

Kowalski S., Lukasiewicz M., Duda-Chodak A., Ziec G. (2013): 5-Hydroxymethyl-2-furfural (HMF) – heat-induced formation, occurrence in food and biotransformation – a review. Polish Journal of Food and Nutrition Sciences, 63: 207–225.

Lichtenberg-Kraag B. (2012): Saccharose degradation over time in stored honey: Influence of time, temperature, enzyme activity and botanical origin. Journal of Food and Nutrition Research, 51: 217–224.

Liu J.R., Ye Y.L., Lin T.Y., Wang Y.W., Peng C.C. (2013): Effect of floral sources on the antioxidant, antimicrobial, and anti-inflammatory activities of honeys in Taiwan. Food Chemistry, 139: 938–943.

Lukasiewicz M., Kowalski S., Makarewicz M. (2015): Antimicrobial an antioxidant activity of selected Polish herbhoneys. LWT-Food Science and Technology, 64: 547–553.

Makhloufi C., Kerkvliet J.D., D'Albore G.R., Choukri A., Samar R. (2010): Characterization of Algerian honeys by palynological and physico-chemical methods. Apidologie, 41: 509–521.

Mandal M.D., Mandal S. (2011): Honey: its medicinal property and antibacterial activity. Asian Pacific Journal of Tropical Biomedicine, 1: 154–160.

Meda A., Lamien C.E., Romito M., Millogo J., Nacoulma O.G. (2005): Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry, 91: 571–577.

Orantes-Bermejo F.J., Torres Fernández-Píñar C. (2009): Evolution of invertase activity in honey from *Castanea sativa* and *Rosmarinus officinalis* collected in Granada. ARS Pharmaceutica, 50: 124–128.

Peter K.V. (ed.) (2012): Handbook of Herbs and Spices. 1st Ed. Oxford, Philadelphia, Woodhead Publishing.

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999): Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26: 1231–1237.

Rybak-Chmielewska H. (2007): Changes in the carbohydrate composition of honey undergoing during storage. Journal of Apicultural Science, 51: 39–48.

Sancho M.T., Muniategui S., Huidobro J.F., Lozano J.S. (1992): Aging of honey. Journal of Agricultural and Food Chemistry, 40: 134–138.

Semkiw P., Skowronek W., Skubida P., Rybak-Chmielewska H., Szczęsna T. (2010): Changes occurring in honey during ripening under controlled conditions based on α-amylase activity, acidity and 5-hydroxymethylfurfural content. Journal of Apicultural Science, 54: 55–64.

Silici S., Sagdic O., Ekici L. (2010): Total phenolic content, antiradical, antioxidant and antimicrobial activities of Rhododendron honeys. Food Chemistry, 121: 238–243.

Silva L.R., Videira R., Monteiro A.P., Valentão P., Andrade P.B. (2009): Honey from Luso region (Portugal): Physicochemical characteristics and mineral contents. Microchemical Journal, 93: 73–77.

Turkmen N., Sari F., Poyrazoglu E.S., Velioglu Y.S. (2006): Effects of prolonged heating on antioxidant activity and colour of honey. Food Chemistry, 95: 653–657.

Received: 2016–09–07
Accepted after corrections: 2017–09–01
Published online: 2017–09–22