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The influence of temperature on the antioxidant potential in different types of honey*

Wpływ temperatury na potencjał antyoksydacyjny różnych rodzajów miodów

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Aim: Honey is the most popular bee product consumed by humans. It is known for its nutritional properties and health benefits, which include neuroprotective effects, the support of the circulatory system, and the beneficial influence it has on skin and respiratory system disorders. The aim of this study was to determine the influence of water temperature used for the preparation of honey solutions on their antioxidant potential.

Material/Methods: Material and methods. The study material included buckwheat honey, black locust honey, and rape honey. Honey solutions (1%) were prepared using distilled water with the temperatures: 25°C, 70°C, 80°C, and 90°C. The antioxidant activity of samples was measured with spectrophotometric method using synthetic radical DPPH.

Results: The antioxidant activity of honey was between 0.29 to 78.50% of DPPH inhibition, depending on the type of honey and the temperature of water used for the preparation of solutions. Buckwheat honey was characterised by the highest antioxidant potential. A significant, directly proportional correlation was observed between the antioxidant potential and the temperature of buckwheat and rape II honey solutions.

Conclusions: In the case of buckwheat honey, rape honey I and black locust honey, the highest antioxidant potential was achieved in solutions prepared using distilled water at 90°C, whereas in the case of rape honey II, the highest values were observed at 80°C and 90°C. The lowest inhibition of the DPPH radical was observed in solutions at 70°C in all of the honey types. In the case of the studied honeys, it is even advisable to prepare water solutions at 80°C or 90°C in order to increase its antioxidant potential, e.g. by adding honey to tea or milk.

Keywords: **honey solutions • temperature • antioxidant activity**

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INTRODUCTION

Honey is the most popular bee product consumed by humans. It is known for its nutritional properties and health benefits. There are many different types of honey on the market, including nectar honey (flower), honeydew honey, and mixed (nectar-honeydew or honeydew-nectar). They are different not only in terms of sensory values, but also in how they influence human health. The most known varieties of honey include rape honey, black locust honey, buckwheat honey, heather honey, lime honey, and polyflora honey. Honey has many health benefits. It has a neuroprotective effect, it supports the functioning of the circulatory system [16, 21], and it helps in the case of respiratory system disorders [12] and skin problems [7]. It is a product with complex chemical content, of which 80% is sugar. Most of the sugar, about 90%, includes glucose and fructose, which are easily assimilated monosaccharides. Oligosaccharides are present in honey in small amounts, but thanks to their prebiotic properties they have a positive influence on the human body [6]. The caloric value of honey in 100g of product is between 320 and 330 kcal. Bee products are not only a rich source of natural nutrients, but they also contain biologically active compounds, including antioxidants [5]. Some of the substances that determine honey's antioxidant potential and play a significant role include: enzymes (e.g. catalase), vitamins (carotenoids, vitamin C, vitamin E), organic acids, amino acids and proteins, as well as numerous polyphenol compounds [32]. In the case of polyphenols, which are considered as the most important antioxidants of honey [15], phenolic acids and flavonoids are worth mentioning. Their amount depends on the botanic origins of honey [29]. The most important quality of antioxidant compounds in food products, including honey, is their ability to inhibit free radical processes. An excessive amount of free radicals, which is referred to as oxidative stress, is associated with such problems as the development of insulin resistance, type 2 diabetes [25], circulatory system diseases, autoagressive disorders, neurodegenerative diseases, and it can also contribute to the process of organism aging and the amplification of carcinogenesis [21]. Therefore, it seems reasonable to search for resources with strong antioxidant properties, which could reduce the formation of free radicals, potentially taking part in the prevention of the aforementioned diseases.

The aim of the study was to determine the influence of the temperature of water used to prepare honey solutions on their antioxidant potential and fluoride content.

MATERIAL AND METHODS

The preparation of honey solutions

The study material included different varieties of honey (buckwheat honey, black locust honey, and two varieties of rape honey) from the West Pomeranian Voivodeship (Poland). Sample of 1.0 g of honeys were weighed and quantitatively transferred to a 100.0 ml measuring flask which was then filled with 25°C, 70°C, 80°C, and 90°C distilled water. The contents were stirred using a magnetic stirrer until a homogeneous solution was achieved. Subsequently, the solution was left to cool down to 25°C. All infusions were prepared in triplicate.

Determining antioxidant activity

The antioxidant activity of samples was measured with spectrophotometric method using synthetic radical DPPH (2,2diphenyl-1-picrylhydrazyl, Sigma). Antioxidant potential (antioxidant activity, inhibition) of tested solutions has been expressed by the percent of DPPH inhibition [33]. The antioxidant potential was determined using UV-Vis spectrophotometer Agilent 8453. All assays were performed in triplicate.

Statistical analysis

In all the experiments, three samples were analysed and all the assays were carried out at least in triplicate. The statistical analysis was performed using Stat Soft Statistica 13.0 and Microsoft Excel 2010. The results are expressed as mean values and standard deviation. To determine the percent of DPPH inhibition, the one-way analysis of variance (ANOVA) and Tukey post-hoc were used. Correlation analysis was performed by Pearson coefficient. Differences were considered significant at $p \leq 0.05$.

RESULTS

The values of antioxidant potential of the studied types of honey, expressed as percent DPPH inhibition of radical, were from 0.29% to 78.50% of DPPH inhibition (Fig. 1). At each temperature, statistically significant differences occurred between the buckwheat honey antioxidant capacity and the others honey solutions ($p = 0.000175$).

For the solution of buckwheat honey, the range of the antioxidant potential was from 33.09% to 78.50% DPPH inhibition (Fig. 2).

The buckwheat honey solution prepared with water at 90°C has the highest antioxidant potential. Differences compared to the remaining solutions were statistically significant (for 25°C and 70°C $p = 0.000174$; for 80°C

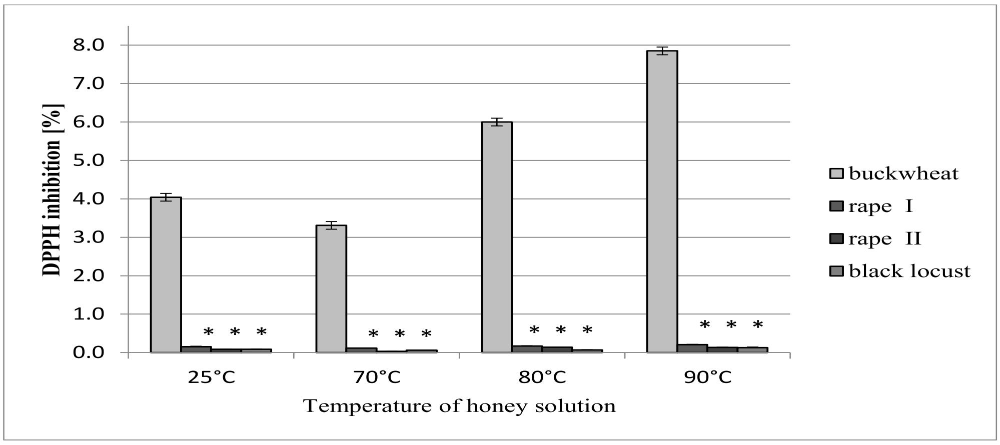

Fig. 1. The antioxidant potential of specific honey types depending on temperature. *p ≤0.05

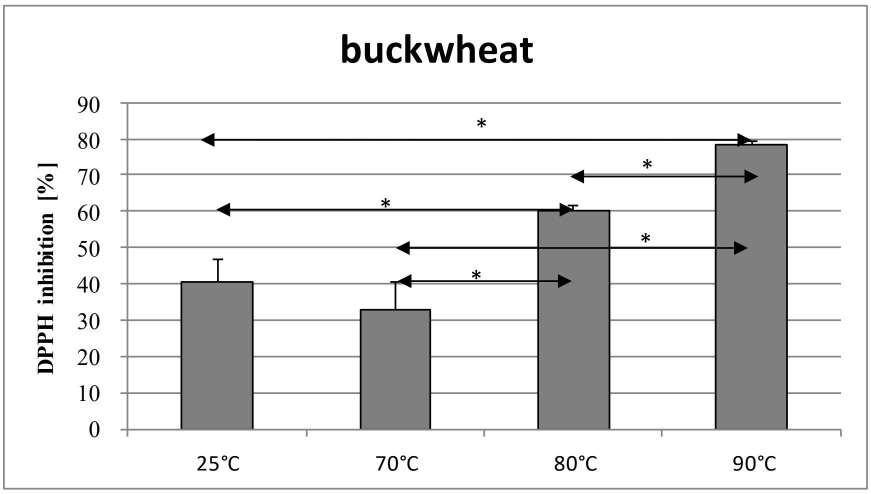

Fig. 2. The antioxidant potential of buckwheat honey solution depending on temperature. *p ≤0.05

p = 0.000194). There was also a statistically significant positive correlation (r = 0.623) between the antioxidant potential of this honey and the temperature of water used to prepare the solutions.

For the solution of rape honey I, the range of the antioxidant potential was from 1.16 to 2.03 % of DPPH inhibition (Fig. 3).

The highest antioxidant potential was found in the solution prepared with water at 90°C. All differences were statistically significant (for 25°C p = 0.000176, for 70°C p = 0.000176, for 80°C p = 0.040155). In the case of solutions of this honey, the relationship between antioxidant potential and temperature was not statistically significant (r = 0.385).

For the solution of rape honey II, the range of the potential was from 0.29 to 1.38 % of DPPH inhibition (Fig. 4).

In the case of rape honey II, the highest antioxidant potential was found in solutions prepared with water at 80°C and 90°C; the difference between these values was not statistically significant (p = 0.852). Between the values characterizing the remaining solutions the differences were statistically significant (p = 0.000175 for all solutions).

For the solution of black locust honey, the range of the potential was from 0.61 to 1.28% DPPH inhibition (Fig. 5).

As for the other honeys, the highest potential was seen in the solution of black locust honey prepared with water at 90°C; differences between this value and the others temperatures were statistically significant (p = 0.000175 for all solutions). The statistically insignificant difference was only between the value for the solution prepared with water at 70°C vs 80°C (p = 0.5690).

In the case of buckwheat honey, rape honey I and black locust honey, the highest antioxidant potential was achieved in solutions prepared using 90°C distilled water, whereas in the case of rape honey II, the highest values were observed at 80°C and 90°C.

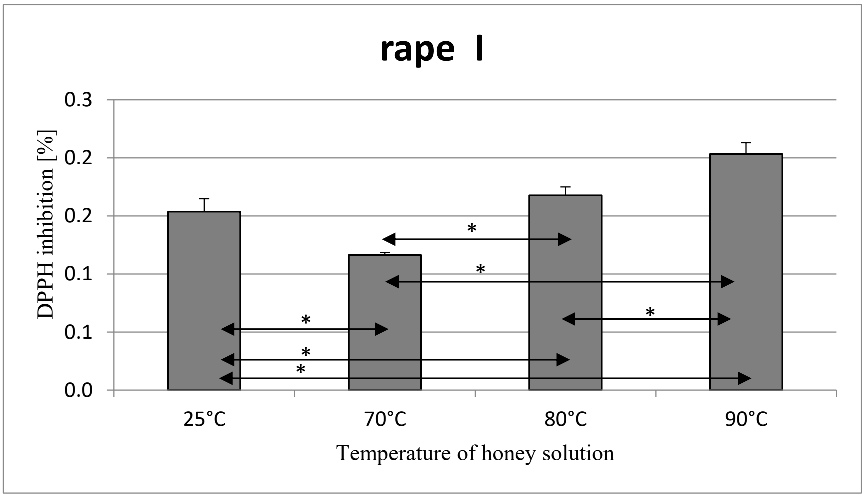

Fig. 3. The antioxidant potential of rape I honey solution depending on water temperature. *p ≤0.05

The lowest inhibition of the DPPH radical was observed in solutions at 70°C in all of the honey types. There is a tendency for antioxidant potential to rise together with the increase in temperature in the range from 70°C to 90°C (especially in terms of buckwheat honey and rape honey I) and to decrease in the range from 25°C to 70°C. In most cases, the differences between results achieved at different temperatures were statistically significant. However, the differences were not significant in the following examples: rape honey II – between temperatures 80°C *vs* 90°C, black locust honey – between temperatures 70°C *vs* 80°C and buckwheat honey – between temperatures 25°C *vs* 70°C.

DISCUSSION

Honey may be considered as a biological indicator of environmental quality and floral biodiversity. Its composition strongly depends on the territory in which it is produced and it is closely tied to the flora visited by bees for its production [14]. In many works, authors point out the influence that the origin of this resource has on the physicochemical properties of honey. The differences were particularly visible in the activities of the enzymes, ash contents, hydroxymethylfurfural (HMF) contents, pH and electrical conductivity.

The values of antioxidant potential of buckwheat honey infusions achieved in the study were similar to those acquired by Majewska et al. [18, 19], who studied buckwheat honey from various regions of Poland. The values were in the range from 29.68% to 75.86% of DPPH inhibition. The study by Wilczyńska showed that the antioxidant properties of buckwheat honey were at the level of 70.6% inhibition [31], in the range from 56.39% to 100% of DPPH inhibition [30]. Hołderna-Kędzia & Kędzia [13] labelled the potential in buckwheat honey achieving 57.6 μg eq. chlorogenic acid/ml. In this type of honey, the value of IC50 achieved by Beretta et al. [3] was 4.0 mg/ml, whereas the value of ORAC measured by Gheldof et al. [11]

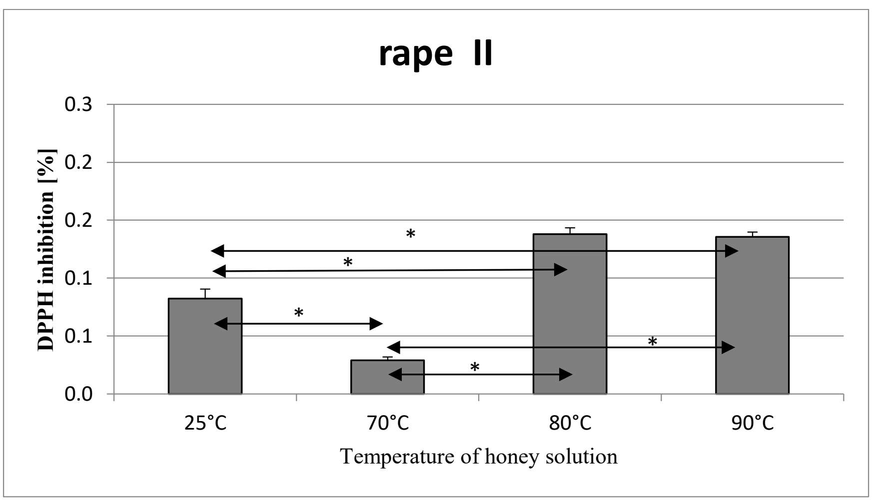

Fig. 4. The antioxidant potential of rape II honey solution depending on water temperature. *p ≤0.05

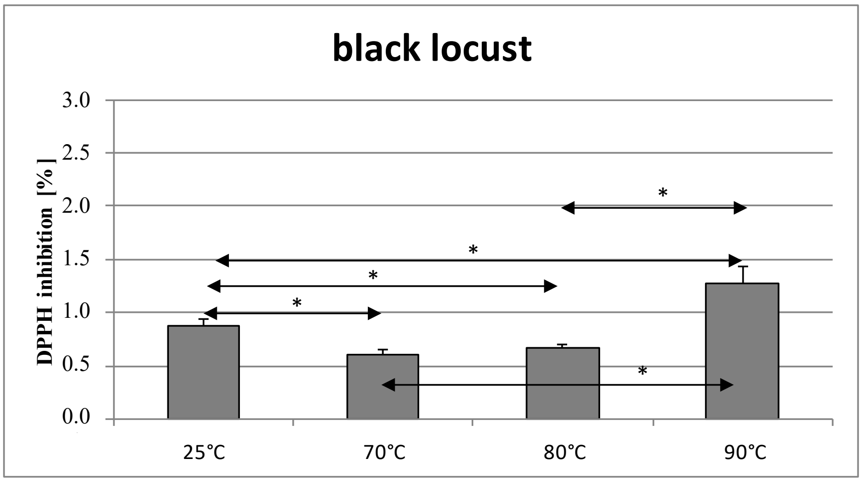

Fig. 5. The antioxidant potential of black locust honey solution depending on temperature. *p ≤0.05

was between 7.47 and 16.95 µmol TE/g. Due to the application of a different unit determining antioxidant potential, it is difficult to compare these results with the ones achieved in our study. Despite this fact, on the basis of these results it can still be concluded that the antioxidant potential of buckwheat honey is from moderate to high. In the case of black locust honey types analysed by Majewska et al. [18], the antioxidant potential was between 7.18% and 13.53% of DPPH inhibition. The results are confirmed by this study. Higher values were achieved by Wilczyńska et al. [31]. The antioxidant potential of black locust honey was in the range between 25.58 and 47%. These differences might have been caused by the differences in the origin of the honey types. There are also other studies concerning the antioxidant properties of black locust honey, which make use of a different unit for this parameter. Chua et al. [8] achieved the value of antioxidant potential at the level of 29.983 mg AAE/100 g, whereas Gheldof et al. [9] noted the value for ORAC at the level of 3 µmol TE/g. In the studies by Hołderna-Kędzia & Kędzia [13], the potential for black locust honey was about 25 µg eq. chlorogenic acid/ml. The value of IC50 achieved by Bertoncelj et al. [4] was 53.8 mg/ml. Similar results were achieved by Šarić et al. [25] and Beretta et al. [2] – in those cases, the values for IC50 were 44.64 mg/ml and 45.45 mg/ml, respectively. In the studies by Meda et al. [20] the value of IC50 indicated stronger antioxidant properties, with the result of 10.53 mg/ml. In comparison to the remaining 27 types of honey studied by these scientists, the result shows that black locust honey has a moderate ability to sweep free radicals. The antioxidant potential of rape types of honey achieved in this study was lower than the values available in literature. In the case of rape honey types, Majewska et al. [19] achieved DPPH inhibition at the level between 28.19% and 44.08%. The antioxidant potential of rape honey according to Wilczyńska was 50.9%[7] and it was in the range from 43.14% to 60.59% [32]. When compared to resources known for having high antioxidant properties, such as green tea [35] or coffee [34], the antioxidant potential of honey solutions is not high, excluding the solutions of buckwheat honey prepared at 80°C and 90°C. However, honey is often used as an addition to tea or herbal infusions and in these cases it can enrich the positive effects of these liquids on human health. Telesiński et al. [27] observed a rise in antioxidant potential of infusions made from hypericum after the addition of black locust honey, whereas Gheldof et al. [10] noticed the same relation between tea and buckwheat honey. The antioxidant potential of the different types of honey analysed in this study can be considered low for black locust honey, moderate for solutions of rape honey and high in relation to some solutions of buckwheat honey. The rising antioxidant potential observed between these honey types (black locust honey < rape honey < buckwheat honey) was also determined by other authors [3, 9, 10, 13, 19, 21, 29, 30, 31]. This fact can be explained by the relation between the colour of honey and its antioxidant potential that was observed by many scientists. It seems that light honey has lower antioxidant potential than dark honey [3, 4, 17, 22, 23, 30]. The results presented in this study confirm the aforementioned observations. Black locust honey, which is considered a light type of honey, had the lowest antioxidant potential, whereas the highest results were achieved in the case of buckwheat honey, which is dark. The content of honey and the associated health benefits can be different depending on the place of origin [30] or the plant species of pollen. Differences can also be the result of the influence of environmental conditions, the applied processing techniques, as well as the time and temperature of storage [2]. Šarić et al. [25] observed that after black locust honey was stored for a year, the value of IC50 rose (44.64 mg/ml before storing, 407.01 mg/ml after one year), which proves that the ability to sweep free radicals became lower. There are not enough studies that would allow for the evaluation of the influence that the preparation temperature of honey solutions has on their antioxidant properties or polyphenol content. Studies carried out on other resources confirm that temperature is an important factor for the parameters mentioned above [1, 33]. Because this relation develops differently depending on the resource, it is difficult to be absolutely clear on the direction it takes. According to Batu et al. [2] and Turkmen et al. [28], the increase in the temperature of storing honey causes a rise in their antioxidant potential, which is confirmed by the observed influence of temperature changes. In this study, the highest value of antioxidant potential was labelled in honeys at 80°C and 90°C. This phenomenon might be associated with the higher kinetic energy of particles (including antioxidants) in the solution, which allows for the acceleration of effective collisions leading to the neutralization of DPPH radical. Another explanation for the rise in antioxidant properties of honey is the creation of antioxidant compounds during the Maillard reaction, which takes place at increased temperatures [28]. At the same time, the observed decrease between room temperature and 70°C may indicate the breakdown of the parts of antioxidant compounds due to increased temperature. The ability of honey to neutralise free radicals is explained by the presence of compounds that have antioxidant properties. The most important compounds include the following: vitamin C, enzymes (catalase, peroxidase, glucose oxidase), phenolic compounds (coumaric acid, chlorogenic acid, quercetin), organic acids (fumaric acid, gluconic acid), carotenoids, free amino acids, proteins, or small amounts of minerals, especially copper and iron [8, 13].

CONCLUSIONS

In the case of buckwheat honey, rape honey I and black locust honey, the highest antioxidant potential was achieved in solutions prepared using 90°C distilled water, whereas in the case of rape honey II, the highest values were observed at 80°C and 90°C. The lowest inhibition of the DPPH radical was observed in solutions at 70°C in all of the honey types. There is a tendency for antioxidant potential to rise together with the increase in temperature in the range from 70°C to 90°C (especially in terms of buckwheat honey and rape honey I) and to decrease in the range from 25°C to 70°C. In most cases, the differences between results achieved at different temperatures were statistically significant. However, the differences were not significant in the following examples: rape honey II – between temperatures 80°C and 90°C, and black locust honey – between temperatures 70°C and 80°C, buckwheat honey - between temperatures 25°C and 70°C.

The study confirmed that the antioxidant potential of water solutions of honey depend on both the type of honey and the temperature of water used to prepare the solutions. Regardless of water temperature, buckwheat honey proved to have the highest antioxidant potential in comparison to other types of honey. A significant directly proportional correlation was found between antioxidant potential and temperatures for buckwheat honey and rape honey II solutions. The studies have proved that high temperature did not always have a negative effect on the health benefits provided by honey. Our studies have shown that the highest antioxidant potential had honey solutions prepared with water at 90°C. In the case of studied honeys, it is even advisable to prepare water solutions at 80°C or 90°C in order to increase its antioxidant potential, e.g. by adding honey to tea or milk.

REFERENCES

[1] Bassani D.C., Nunes D.S., Granato D.: Optimization of phenolics and flavonoids extraction conditions and antioxidant activity of roasted yerba-mate leaves (*Ilex paraguariensis* A. St.-Hil., *Aquifoliaceae*) using response surface methodology. An. Acad. Bras. Cienc., 2014; 86: 923–933

[2] Batu A., Aydoğmuş R.E., Bayrambaş K., Eroğlu A., Karakavuk E., Eroğlu Z.: Changes in Brix, pH and total antioxidants and polyphenols of various honeys stored in different temperatures. J. Food Agric. Environ., 2014; 12: 281–285

[3] Beretta G., Granata P., Ferrero M., Orioli M., Facino R.M.: Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Anal. Chimica Acta, 2005; 533: 185–191

[4] Bertoncelj J., Doberšek U., Jamnik M., Golob T.: Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chem., 2007; 105: 822–828

[5] Bobiş O., Mărghitaş L.A., Dezmirean D., Bonta V., Mihai C.M.: Beehive products: source of nutrients and natural biologically active compounds. J. Agroaliment. Proc. Technol., 2010; 16: 104–109

[6] Borawska J., Bednarski W., Gołębiewska J.: Profile of honey saccharides and possibilities of applying Bifidobacterium to modify their composition and properties. Food, Science, Technologies, 2011; 3: 29–39

[7] Burlando B., Cornara L.: Honey in dermatology and skin care: a review. J. Cosmet. Dermatol., 2013; 12: 306–313

[8] Chua L.S., Rahaman N.L., Adnan N.A., Tan T.T.: Antioxidant activity of three honey samples in relation with their biochemical components. J. Anal. Methods. Chem., 2013; 2013: 313798

[9] Gheldof N., Engeseth N.: Antioxidant capacity of honeys from various floral sources *based* on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. J. Agric. Food Chem., 2002; 50: 3050–3055

[10] Gheldof N., Wang X.H., Engeseth N.J.: Identification and quantification of antioxidant components of honeys from various floral sources. J. Agric. Food Chem., 2002; 50: 5870–5877

[12] Henatsch D., Wesseling F., Kross K.W., Stokroos R.J.: Honey and beehive products in otorhinolaryngology: a narrative review. Clin. Otolaryngol., 2016; 41: 519–531

[13] Hołderna-Kędzia E., Kędzia B.: Research on an antioxidant capacity of honeys. Acta Agrobotan., 2006; 59: 265–269 (in Polish)

[14] Ioannidou M.D., Zachariadis G.A., Anthemidis A.N., Stratis J.A.: Direct determination of toxic trace metals in honey and sugars using inductively coupled plasma atomic emission spectrometry. Talanta, 2005; 65: 92–97

[15] Kesić A., Crnkić A., Ibrišimović-Mehmedinović N., Šestan A., Ćatović B.: Changes of antioxidant activity in honey as a result of Haber-Wais reaction. Am. J. Applied Chem., 2014; 2: 112–116

[16] Khalil M.I., Sulaiman S.A.: The potential role of honey and its polyphenols in preventing heart diseases: A review. Afr. J. Tradit. Complement. Altern. Med., 2010; 7: 315–321

[17] Lachman J., Hejtmánková A., Sýkora J., Karban J., Orsák M., Rygerová B.: Contents of major phenolic and flavonoid antioxidants in selected Czech honey. Czech J. Food Sci., 2010; 28: 412–426

[18] Majewska E.: Comparison of chosen properties of bright and dark honeys. Science, Nature, Technologies, 2009; 13: 1–9 (in Polish)

[19] Majewska E., Kowalska J., Drużyńska B., Derewiaka D., Ciecierska M.: The study of correlation between total poliphenol content and the capacity to deactivate the DPPH radicals in selected honeys. Aparatura Badawcza i Dydaktyczna, 2014; 2: 127–133 (in Polish)

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

[21] Pham-Huy L.A., He H., Pham-Huy C.: Free radicals, antioxidants in disease and health. Int. J. Biomed. Sci., 2008; 4: 89–96

[22] Piljac-Žegarac J., Stipčević T., Belščak A.: Antioxidant properties and phenolic content of different floral origin honeys. J. Api. Prod. Api. Med. Sci., 2009; 1: 43–50

[23] Pontis J.A., da Costa L.A., Da Silva S.J., Flach A.: Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. Food Sci. Technol., 2014; 34: 69–73

[24] Rahman M.M., Gan S.H., Khalil M.I.: Neurological effects of honey: Current and future prospects. Evidence-Based Compl. Alternative Med., 2014; 2014: 958721

[25] Šarić G., Marković K., Major N., Krpan M., Uršulin-Trstenjak N., Hruškar M., Vahčić N.: Changes of antioxidant activity and phenolic content in acacia and multifloral honey during storage. Food Tech. Biotech., 2012; 50: 434–441

[26] Tangvarasittichai S.: Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World J. Diabetes, 2015; 6: 456–480

[27] Telesiński A., Grzeszczuk M., Pobłocki J., Jankowski J., Jadczak D., Zakrzewska H.: Antioxidant activity of some galenic medicines prepared from st. John's wort (*Hypericum perforatum* L.) with added honey. Brom. Chem. Toksykol., 2012; 45: 1227–1232 (in Polish)

[28] Turkmen N., Sari F., Poyrazoglu E.S., Velioglu Y.S.: Effects of prolonged heating on antioxidant activity and colour of honey. Food Chem., 2006; 95: 653–657

[29] Vit P., Rodríguez-Malaver A., Rondón C., González I., Di Bernardo M.L., García M.Y.: Bioactive indicators related to bioelements of eight unifloral honeys. Arch. Latinoamer. Nutr., 2010; 60: 405–410

[30] Wilczyńska A.: Changes in colour and antioxidant activity of stored honeys. Brom. Chem. Toksykol., 2011; 44: 945–950 (in Polish)

[31] Wilczyńska A.: An attempt to use linear regression to determine the relationship between color and antioxidant properties of honey. Sci. J. Gdynia Maritime Univ., 2013; 80: 13–19 (in Polish)

[32] Wilczyńska A., Przybyłowski P.: Characterization of honey's polyphenols. Sci. J. Gdynia Maritime Univ., 2009; 61: 33–38 (in Polish)

[33] Wolska J., Czop M., Jakubczyk K., Janda K.: Influence of temperature and brewing time of nettle (*Urtica dioica L.)* infusions on vitamin C content. Rocz. Panstw. Zakl. Hig., 2016; 67: 367–371

[34] Wolska J., Janda K., Jakubczyk K., Szymkowiak M., Chlubek D., Gutowska I.: Levels of antioxidant activity and fluoride content in coffee infusions of arabica, robusta and green coffee beans in according to their brewing methods. Biol. Trace Elem. Res., 2017; 179: 327–333

[36] Zych I., Krzepiłko A.: Measurement of total antioxidant capacity of selected antioxidants and infusions using DPPH radical reduction. Chemia, Dydaktyka, Ekologia, Metrologia, 2010; 15: 51–54 (in Polish)

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