Studies on Physicochemical Properties of Selected Apiproducts as Materials for Preparation of Protein Spheres

Sonia KUDŁACIK-KRAMARCZYK, Magdalena GŁĄB, Anna DRABCZYK, Bożena TYLISZCZAK, Henryk KOŁOCZEK

Cracow University of Technology, Faculty of Engineering and Chemical Technology, Warszawska 24 Str. 31-155 Cracow, Poland; emails: skudlacik@chemia.pk.edu.pl, magdalenaglab@op.pl, adrabczyk@chemia.pk.edu.pl, btyliszczak@chemia.pk.edu.pl, koloczek@chemia.pk.edu.pl

http://doi.org/10.29227/IM-2019-01-23

Submission date: *11-07-2018* | Review date: *02-04-2019*

Abstract

The main purpose of this work was to determine the impact of the location of bee apiaries in different parts of Poland on the physicochemical properties of selected bee honeys. Studies focused on the determination of the antioxidant properties of apiproducts. The water content in honeys as well as their pH values were also analyzed. Four types of honeys (honeydew, linden, buckwheat, acacia) originating from various parts of Poland were selected for the research. Based on the obtained results, it can be stated that the examined honeys differed in physicochemical properties, which was related to the differences in their chemical composition. All honeys tested showed antioxidant properties. It can also be found that the water content in analyzed products was related to their origin and variety. Analyzing pH measurements, it can also be observed that all tested samples were characterized by acidic properties.

Keywords: bee honey, antioxidant activity, DPPH, UV-VIS spectroscopy

Introduction

Currently, development of new drug carriers is observed. However, apart from efficiency of such drug systems it is also important to create such carriers that after release of drug will degrade into non-toxic substances that will not have a negative impact on environment. The toxic remains of applied carriers can be released during its degradation and can induce a cytotoxic effect. Therefore, a main objective of the presented research is to create an innovative carriers based on proteins derived from apiary products. Such a carrier will be modified using active substances such as anticancer drug. This solution will also affect the improvement of cancer treatment.

According to data from the National Cancer Registry over the past three decades, the number of cancer cases has doubled. Therefore scientists are looking for solutions that will improve the effectiveness of cancer treatment. In the standard methods of administering cancer drugs, their therapeutic possibilities are not fully exploited. Additionally, traditional methods of treatment often cause undesirable side effects. Moreover, in traditional treatment, the healing substance affects the entire body, and only a small part of it goes to the place affected by the disease or inflammation. Therefore intensive research conducted by scientists on the improvement of treatment methods led to the formation of carriers with specific properties. Due to the need of preparation of new, more effective drug delivery systems, the main objective of the research involves the development of innovative drug carriers based on proteins derived from apiary products. Such a carrier is characterized by a better ability to bind and release the drug compared to other carriers, degrades after fulfilling its role and is not toxic to the patient. Preparation of carriers has a low energy demand. There is no need of use of toxic reagents and any waste are not generated. Therefore two main advantages will be achieved: eco-friendly process of preparation as well as biodegradability of prepared carriers that don't affect adversely on the environment. The development of technology for the preparation of drug carriers based on proteins derived from apiary products is characterized by a high application potential.

Bee honeys from various parts of Poland and from various manufacturers constitute the research material for formation the above-mentioned carriers. The preliminary analysis of the physico-chemical properties of this material is necessary to select the appropriate honey for isolation of the protein fractions from it and to prepare polymer carriers. In this paper, description and analysis of the results of research works on selected bee honeys is resented. The following analyzes were conducted: refractometric determination of water content in selected apiproducts on the example of mel, analysis of the impact of honey type and its origin on water content, determination of honey antioxidative properties by spectroscopic technique using a DPPH radical (2.2 - diphenyl-1-picrylhydrazyl) and pH measurements of selected bee honeys over several weeks to check the impact of geographic origin and type of honey on pH values. During the measurements, the influence of the storage time of honeys on their pH values was also determined.

Materials and methods

In order to compare properties of selected apiproducts four types of honeys deriving from different parts of Poland were selected. Their type and origin is summarized in Table 1.

All chemicals applied in the research were at least of analytical reagent grade. DPPH and methanol were supplied by Sigma Aldrich.

Tab. 1. Types of analyzed honeys
Tab. 1. Rodzaje analizowanych miodów

Manufacturer	Region of Poland	Type of honey
Kosecki	North	Honeydew Acacia Buckwheat Linden
Gładys	Center	
Kószka	South	
Dutkowiak	West	

Tab. 2. Measurements of water content in honeys from the Kosecki company
Tab. 2. Pomiary zawartości wody w miodzie firmy Kosecki

Type of honey	Water content [%]		
	Measurement I	Measurement II	Measurement III
Honeydew	18.6	17.1	17.2
Buckwheat	19.5	18.1	17.9
Linden	18.5	17.3	17.2
Acacia	16.9	16.0	15.9

Tab. 3. Measurements of water content in honeys from the Gładys company
Tab. 3. Pomiary zawartości wody w miodzie firmy Gładys

Type of honey	Water content [%]		
	Measurement I	Measurement II	Measurement III
Honeydew	15.8	15.8	15.9
Buckwheat	18.1	18.2	19.0
Linden	17.8	17.9	18.2
Acacia	17.2	17.1	17.0

Tab. 4. Measurements of water content in honeys from the Dutkowiak company
Tab. 4. Pomiary zawartości wody w miodzie firmy Dutkowiak

Type of honey	Water content [%]		
	Measurement I	Measurement II	Measurement III
Honeydew	17.4	17.1	17.0
Buckwheat	18.4	18.4	18.5
Linden	18.1	18.0	18.3
Acacia	17.1	17.0	16.5

Tab. 5. Measurements of water content in honeys from the Kószka company
Tab. 5. Pomiary zawartości wody w miodzie firmy Kószka

Type of honey	Water content [%]		
	Measurement I	Measurement II	Measurement III
Honeydew	17.2	17.2	18.1
Buckwheat	17.8	17.7	18.2
Lindem	17.4	17.6	18.1
Acacia	17.9	17.8	18.6

Refractometric analysis of water content

The RHN1-ATC refractometer was used in the research. Before proceeding to the proper analysis of the water content, the device was calibrated using a calibration solution. Then, sample of analyzed honey was placed between two prisms and the refractometer was set towards daylight. After stabilization of the dividing line, the water content was read from the scale. Three measurements were conducted for each type of honey at 3-week intervals.

Analysis of pH values

The Multifunction meter CX - 701 multifunction apparatus was used for analysis. A sample of honey was placed in a clean container, and then the electrode of the device was immersed in it. Three measurements were made at 4-week intervals.

Analysis of antioxidant properties

During the study, analysis by means of the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical in the form of an alcoholic solution was applied. The solution is characterized by a dark violet color with a maximum absorbance at a wavelength λ = 517 nm. The reaction scheme is peresented above.

In reaction with an antioxidant, DPPH captures electrons, forms a reduced form, and converts to a slightly yellow prod-

Tab. 6. Results of pH measurements for honeys from Kosecki
Tab. 6. Wyniki pomiarów pH dla miodów od Koseckiego

Type of honey	pH value		
	Measurement I	Measurement II	Measurement III
Honeydew	4.769	4.756	4.547
Buckwheat	3.866	3.873	3.595
Linden	4.090	4.094	3.724
Acacia	4.968	4.574	3.724

Tab. 7. Measurements of water content in honeys from the Kosecki company
Tab. 7. Pomiary zawartości wody w miodzie firmy Kosecki

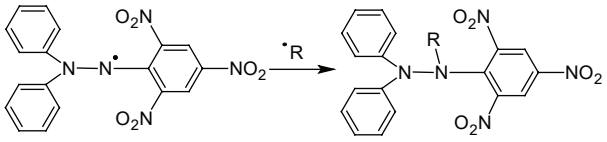
Type of honey	pH value		
	Measurement I	Measurement II	Measurement III
Honeydew	4.989	4.967	4.715
Buchwheat	4.014	3.832	3.540
Linden	4.415	4.282	3.910
Acacia	4.681	4.424	4.042

Tab. 8. Measurements of water content in honeys from the Gładys company
Tab. 8. Pomiary zawartości wody w miodzie firmy Gładys

Type of honey	pH value		
	Measurement I	Measurement II	Measurement III
Honeydew	4.606	4.615	4.600
Buckwheat	3.612	3.743	3.521
Linden	3.828	3.973	3.745
Acacia	4.692	3.690	3.941

Tab. 9. Results of pH measurements for honeys from Kószka
Tab . 9. Wyniki pomiarów pH dla miodów z Kószka

Type of honey	pH value		
	Measurement I	Measurement II	Measurement III
Honeydew	4.556	4.630	4.621
Buckwheat	3.863	3.692	3.725
Linden	3.925	4.080	3.950
Acacia	3.600	3.645	3.695



uct. These changes were monitored spectrophotometrically using a Biosens Spectrophotometer V-5100.

Results and discussion

Refractometric analysis of water content

Results of measurements of water content in honeys deriving from different parts of Poland are presented in Tables 2.–5. Three measurements at 3-week intervals were performed for each sample.

The percentage of water content in the tested honey samples ranges from 15.8% to 19.5%. The highest value was recorded for buckwheat honey from Kosecki, while the least amount of water contained honeydew honey from Gładys. The average water content in all honey was 17.6%. According to the Ordinance of the Minister of Agriculture and Rural Development, which specifies the requirements for the commercial quality of honey, the water content in nectar honey may not exceed 20%. For all tested samples, values below 20% were obtained.

In the case of honeys deriving from Dutkowiak, Kosecki and Gładys, the percentage of water varies depending on the variety of the tested honey. These differences are influenced by many factors, including a different source of floral nectar and the origin of honeys from different regions of the country, and thus other climatic conditions prevailing during the harvest. This dependence was not observed in case of honeys deriving from Kószka - similar values were obtained for each of the four types of honeys. However, honeys produced from the nectar of different flower species are characterized by different composition, therefore it is unlikely that such results were obtained. It can therefore be assumed that the original water content in these honeys exceeded certain norms and

Tab. 10. Antioxidant activity of analyzed honeys
Tab. 10. Aktywność przeciwutleniająca analizowanych miodów

Absorbance of control sample	Absorbance of tested sample	Manufacturer of analyzed honey	Type of honey	Antioxidant activity (A) [%]
0.6745	0.5452	Dutkowiak	Acacia	19.17
	0.5282	Gładys		21.69
	0.5868	Kosecki		13.00
	0.6454	Kószka		4.31
	0.3844	Dutkowiak	Buckwheat	43.01
	0.4848	Gładys		28.12
	0.3325	Kosecki		50.70
	0.5955	Kószka		11.71
	0.4793	Dutkowiak	Linden	28.94
	0.5212	Gładys		22.73
	0.4385	Kosecki		34.99
	0.5503	Kószka		18.41
	0.2144	Dutkowiak	Honeydew	68.21
	0.4361	Gładys		35.34
	0.3027	Kosecki		55.12
	0.4387	Kószka		34.96

it was intentionally changed to the value consistent with the regulation.

The water content for all honey changed over time. Differences were small, and way of sampling of honeys for testing could affect the change in the water content of the tested samples, because honey is characterized by hygroscopic properties, therefore in the case of exposure to the environment, some water could evaporate when the air was dry or absorbed in the case of space with high humidity. Such compounds as fructose (main sugar components of honey) present in honey are responsible for these properties. The fructose content depends on the honey variety, which affects different hygroscopic properties of the tested samples.

Measurements of pH values of analyzed honeys

Results of measurements of pH values of all analyzed honeys are presented below. Three measurements were carried out at 4-week intervals.

The pH values of the analyzed honeys are in the range from 3.521 to 4.989. It can therefore be concluded that all tested honey samples exhibit acidic properties. The lowest pH value was recorded for buckwheat honey from Dutkowiak, while the highest one was measured in case of honeydew honey from Gładys. The low pH of honey is mainly due to the presence of organic acids such as citric, gluconic and formic in analyzed honeys.

According to the obtained pH results, the lowest values were observed in case of the group of buckwheat honeys. All four samples, regardless of the manufacturer, were characterized by the lowest pH and thus the highest content of above-mentioned acids. This is probably related to the chemical composition of nectar obtained from buckwheat. On other hand, the highest pH values were recorded for honeydew honeys, while intermediate values for acacia and linden honeys. In the case of the majority of tested honeys, the decrease of the pH values over time was observed. The increasing acid reaction may result from the biochemical processes taking place in the product. The most important one is the process of oxidation of glucose (1) present in honey, proceeding in accordance with the following reaction:

$$C_6H_{12}O_6 + O_2 + H_2O \rightarrow C_6H_{12}O_7 + H_2O_2 \quad (1)$$

This process is related to the activity of the enzyme - glucose oxidase, which oxidizes glucose to the gluconic acid and H2O2. As a result of the reaction gluconic acid is formed, thus an increase in the amount of acids in the honey is observed, and therefore its lower pH value. As a result of a fact that honeys of different types originating from different parts of Poland are characterized by different enzymatic activity, differences in the dependence of pH decrease on storage time can be observed.

Analysis of antioxidant activity

Values of antioxidant activities of analyzed honeys were calculated using the following formula:

$$A = \frac{A_k - A_b}{A_k} \times 100\%$$

where:
A – antioxidant activity,
A_k – absorbance of control sample,
A_b – absorbance of analyzed sample.

The following measurement results were used for the calculations, taking into account the maximum absorbance value of the control sample and the test sample (measurement after 2 h and 15 min from the beginning of the reaction). The table below presents the calculated values of antioxidant activity.

Antioxidative activity of analyzed honeys ranged from 3.14% to 68.21%. The greatest efficiency of free radicals scavenging was determined for honeydew honey from Dut-

kowiak, while the smallest value was noted for the acacia honey from the Kószka company.

The obtained results can be analyzed in two ways - due to the producer and taking into account different varieties of honeys. In the first case, the best results were obtained for honeys from Dutkowiak, while the worst for honeys from Kószka, for which the antioxidant activity of any tested honey did not exceed 35%. The different properties of honeys from various producers are probably related to the various method of honey production and its storage. The origin of honey, that is the location of the apiary, is also important. Dutkowiak honey, which showed greater antioxidant capacity, came from the western part of Poland, whereas honey from Kószka from the south of the country.

Considering the different varieties of honeys, honeydew honeys proved to be the best in terms of antioxidant activity. Acacia honeys were characterized by the smallest values, in this group the maximum activity was 21%, while in the honeydew group it was 68%. Honeydew, buckwheat, linden and acacia honeys differ in their chemical composition, and thus have a different content of phenolic compounds and enzymes responsible for antioxidant properties.

Conclusion

Based on the conducted studies it can be concluded that the location of bee apiaries affects the physicochemical properties of selected varietal honeys, since the same variety of honey originating from different parts of the country in some cases has significantly different properties. Honey of different varieties are characterized by different physicochemical properties, i.e. different water content, different pH values and different antioxidant activity. The water content for all tested honeys meets the requirements relating to the commercial quality of honey, as the pH values of the tested honeys are in the range from 3.521 to 4.989.

Summing up the above conclusions, it can be concluded that the physicochemical properties of honey depend on both their variety, and hence the source of the floral nectar, on the producer and associated apiary location as well as on the method of production and storage of the apiproduct. Such material can certainly be used for the preparation of protein spheres, which in the current growth of medicine may give a broader look at the aspect of anti-cancer therapy. The fact of the properties of such carriers, depending on the proteins isolated from a given variety of honey, also seems to be very interesting.

Literatura – References

1. NELSON, Eino et al. Kinetics of drug absorption, distribution, metabolism, and excretion. Journal of Pharmaceutical Sciences, 50 (3), 1961, p. 181–92.
2. MOUT, Rubul et al. Surface functionalization of nanoparticles for nanomedicine. Chemical Society Reviews, 41 (7), 2012, p. 2539 - 2544.
3. ELSABAHY, Mahmoud et al. Design of polymeric nanoparticles for biomedical delivery applications. Chemical Society Reviews, 41 (7), 2012, p. 2545 - 2561.
4. CURNIS, Flavio et al. Improving chemotherapeutic drug penetration in tumors by vascular targeting and barrier alteration. Journal of Clinical Investigation, 110 (4), 2002, p. 475–82.
5. BRUNNER, Anette et al. pH and osmotic pressure inside biodegradable microspheres during erosion. Pharmaceutical Research, 16 (6), 1999, p. 847–53.
6. MIZRAHI, Avshalom et al. Bee Products, Properties, Applications, and Apitherapy. Springer, New York, 1997.
7. MORITA, Hiroyuki et al. Effect of royal jelly ingestion for six months on healthy volunteers. Nutrition Journal, 11, 2012, p. 77 – 82.
8. LOHCHAROENKAL, Warangkana et al. Protein Nanoparticles as Drug Delivery Carriers for Cancer Therapy. BioMed Research International, 2014, 2014, p. 1-12.

Badania właściwości fizykochemicznych wybranych pszczelich produktów ubocznych jako materiałów do wytwarzania sfer białkowych

Głównym celem tej pracy było określenie wpływu lokalizacji pasiek pszczelich w różnych częściach Polski na właściwości fizykochemiczne wybranych miodów pszczelich. Badania koncentrowały się na określeniu właściwości przeciwutleniających produktów api (pszczelich) . Analizowano również zawartość wody w miodzie, a także ich wartości pH. Do badań wybrano cztery rodzaje miodów (spadziowy, lipowy, gryczany, akacjowy) pochodzące z różnych regionów Polski. Na podstawie uzyskanych wyników można stwierdzić, że wytwarzane miody różniły się właściwościami fizykochemicznymi, co było związane z różnicami w ich składzie chemicznym. Wszystkie badane miody wykazały właściwości przeciwutleniające. Można również stwierdzić, że zawartość wody w analizowanych produktach była związana z ich pochodzeniem i odmianą. Analizując pomiary pH, można również zauważyć, że wszystkie badane próbki charakteryzowały się właściwościami kwasowymi.

Słowa kluczowe: miód pszczeli, aktywność antyoksydacyjna, DPPH, spektroskopia UV-VIS