

TOTAL PHENOLIC CONTENT AND ANTIRADICAL ACTIVITY OF HONEY POWDERS

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Abstract

Honey powders could be an innovative and attractive substitutes to liquid honey. The production of honey powders would allow to expand honey's usage in food industry, cosmetics and pharmaceuticals. In this research, freeze drying method was performed to obtain honey powder samples. Overall, 4 different formulations were prepared to obtain the powder samples. The aim of the research was to determine the total phenolic content and antiradical activity of the freeze-dried honey powder samples by spectrophotometric method. The content of total phenolics in the samples ranged from 92 to 146 mg GAE 100 g⁻¹ of dry matter. Antiradical activity was measured with DPPH radical scavengers, and the IC₅₀ results ranged from 6 to 12 mg mL⁻¹.

Key words: honey powder, freeze drying, antiradical activity, total phenolic content.

Abbreviations: H, honey; MD, maltodextrin; MN, mannitol; DPPH, 2,2-diphenyl-1-picrylhydrazyl; IC₅₀, half maximal inhibitory concentration; AA, antiradical activity; GAE, gallic acid equivalent.

Introduction

Honey is a naturally sweet food stuff, which has high nutritional value and it's therapeutic properties are well-known over the centuries. Honey consists of 200 chemical compounds (Bueno-Costa *et al.*, 2016). The wide range of different substances are found in honey such as enzymes, amino acids, organic acids, monosaccharides, different phenolic compounds (Tezcan *et al.*, 2011; Da Silva *et al.*, 2016). Noteworthy that the chemical profile of honey depends on many factors, for instance, climate, botanical and geographical origin. Honey is classified in different types, which are based on their geographical origin or floral source (Sakač *et al.*, 2018). The phenolic profile of honey is mostly the same, but the concentrations of phenolic compounds in different type are variant (Badolato *et al.*, 2017). Phenolic compounds such as phenolic acids and flavonoids are natural antioxidants (da Silva *et al.*, 2013). Antioxidants have a significant role in human health (Mithul Aravind *et al.*, 2021), they protect the body cells from free radicals. Free radicals are very reactive and have a tendency to react with biomolecules, which could result in serious cell damages (Zheng *et al.*, 2020).

The production of powdered honey is an innovative approach to expand the application of honey in food industry, pharmaceutical industry. Longer shelf life and expanded usage in food processing (Tong *et al.*, 2010; Kılınc & Demir, 2017; Tomczyk *et al.*, 2020) are a few of advantages of powdered honey, which present powdered honey as an attractive substitute to natural honey. The interest of production of honey powder among food scientists has been increased (Samborska, 2019). Different drying methods (Nurhadi *et al.*, 2012; Sramek *et al.*, 2016; Samborska *et al.*, 2017) have been studied for obtaining honey powder. As honey is a sugar-rich food product (Muzaffar, 2015), its drying is very challenging. The main issue is that sugar-rich

food products during drying can make lumps or syrup (Bhandari *et al.*, 1997). This issue mainly can be prevented by adding different drying aids or carriers to increase the glass temperature of drying particles (Shi *et al.*, 2013). Freeze-drying among all drying methods, which are recently performed for obtaining high quality honey powder, is not very common (Ganaie *et al.*, 2021). The main reason of that is a long production time, which expands the cost of production. Nevertheless, freeze-drying is bioactive compounds preserving drying technique (Ranieri *et al.*, 2017; Prosapio & Norton, 2018).

The phenolic profile of honey and other food products can be evaluated by different analytical techniques (Chan *et al.*, 2013; Zhu *et al.*, 2019; Tian *et al.*, 2021). Spectrophotometric method is one of the most popular analytical methods due to its low cost, simplicity, rapidness. Total phenolics content is commonly determined using Folin-Ciocalteu reagent. The method is easy to modify and allows to detect total phenolic content in a wide range of products (Kek *et al.*, 2014; Paradiso *et al.*, 2016; Hinojosa-Nogueira *et al.*, 2020). Antiradical activity of different food products is examined by the DPPH test (Marinova & Batchvarov, 2011; Naji *et al.*, 2020).

The production of honey powder involves thermal treatment and could negatively affect the quality of honey by, for example, decreasing enzyme activity (Tosi *et al.*, 2008; Sramek *et al.*, 2016; Samborska *et al.*, 2017), increasing the concentration of 5-hydroxymethylfurfural (Pasiak *et al.*, 2017; Kanar & Mazl, 2019).

As phenolic compounds are sensitive to temperature changes (Molaveisi *et al.*, 2019; Samborska *et al.*, 2019), it is important to examine honey powder. The aim of the research was to determine the total phenolic content and antiradical activity of the freeze-dried honey powder samples by spectrophotometric method.

Materials and Methods

The experimental work of study took place at the Scientific laboratory of Natural compounds at the Department of Chemistry, Faculty of Food Technology, Latvia University of Life Sciences and Technologies.

Buckwheat honey was purchased from a local beekeeper. Buckwheat honey was harvested in 2019. The beehives were placed in Jelgava district, the southern-central part of Latvia.

Maltodextrin (STAR-DRI® 10 NG, TALE & LYLE, USA) was delivered by Latvian company (BANG & BONSOMER LATVIA, Rīga, Latvia). Dextrose equivalent (DE) of maltodextrin was 10.4. Moisture content, according to the certificate given by the company, was 4.3%. Mannitol was purchased from Riedel-de Haën and moisture content was 0.5%. (Honeywell, Germany).

Freeze-drying of honey solution

Four different 30% aqueous solutions of honey with mannitol and maltodextrin were prepared for the experiments.

The proportions of honey and carriers in feed solutions are shown in Table 1.

Table 1

Proportion of honey and carrier in feed solution

Carrier	Ratio (honey: carrier)
Mannitol	1:1
Maltodextrin	1:1
Mannitol	1:2
Maltodextrin	1:2

The solutions were poured into plastic freezer containers. The height of solution layer in each container was approximately one centimetre. The containers were kept in the freezer at -20 ± 1 °C for 3 hours. Drying of frozen samples were performed as described by Keke (Keke & Cinkmanis, 2020). The obtained freeze-dried honey samples each were grinded into powder by porcelain mortar and pestle. The powder samples were collected in polyethylene bags and stored at $+20$ °C temperature in dark and dry place until further analysis.

Detection of moisture content

Determination of moisture content was carried out using a moisture analyzer AND MX-50 (A&D Company, Limited, Japan). The moisture content of samples were evaluated using the method described by Keke (Keke & Cinkmanis, 2020). The software 'WinCT-Moisture' was used for the data recording.

Detection of total phenolics

The content of total phenolics of the obtained honey powders and buckwheat honey were evaluated

by the Folin-Ciocalteu method. The honey powder samples and honey were dissolved in deionized water. The concentration of each prepared solutions was 0.1 g mL^{-1} . Then 2.5 mL of 0.2 M Folin-Ciocalteu reagent (Sigma Aldrich®, Germany) were added to 0.5 mL of prepared solutions and mixed. After 5 minutes 2 mL of 7.5% Na_2CO_3 solution were added to the mixture and mixed together. The reaction solution was stored at $+20$ °C temperature in the dark for 2 hours. After incubation the content of total phenolics was determined by spectrophotometer Jenway 6405 UV/Vis (JENWAY, the U.K.). The absorbance of the samples was measured at 760 nm. Deionized water was used as blank solution. The results were expressed as gallic acid equivalents $\text{mg GAE } 100 \text{ g}^{-1}$ dry matter (Cinkmanis, Dimins, & Mikelsone, 2017).

Detection of antiradical activity

The antiradical activity of the samples was determined using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The obtained powders and buckwheat honey were dissolved in deionized water at concentrations ranging from 2 to 14 mg mL^{-1} . And 3.5 mL of prepared solution was well-mixed with 1.5 mL of 0.1 mM DPPH (Sigma Aldrich®, Germany) solution. The reaction solution was stored at $+20$ °C temperature in the dark for 30 minutes. After incubation the determination of antiradical activity was performed by spectrophotometer Jenway 6405 UV/Vis (JENWAY, the U.K.). The absorbance of the samples was measured at 515 nm. The results were expressed as the percentage of antiradical activity (AA). The antioxidant activity was calculated according to the following formula:

$$AA = \left(\frac{ABS_{control} - ABS_{sample}}{ABS_{control}} \right) \times 100 \quad (1)$$

As blank solution, dissolved honey powder solutions at the same concentrations as previously were described and 3.5 mL of methanol were used. The control solution consisted of 3.5 mL of methanol (Sharlab S.L., Spain) and 1.5 mL of 0.1 mM DPPH solution. The IC_{50} values were calculated by linear regression of plots, where the concentration of prepared solutions were represented on the abscissa, and the antiradical activity of prepared solutions was represented on the ordinate (Pontis *et al.*, 2014; Cinkmanis, Dimins, & Mikelsone, 2017).

Statistical analysis

The assays were repeated three times, and the results were expressed as mean values \pm standard deviation (SD).

One-way analysis of variance (ANOVA) and Tukey's test ($p < 0.05$) were used to verify statistical differences. Analysis were performed by Microsoft 365 Excel.

Table 2

Moisture content and IC₅₀ value in the analysed samples

Carrier	Ratio (honey: carrier)	Moisture content, %	IC ₅₀ , mg mL ⁻¹
Mannitol	1:1	4.5±0.2	6±1
	1:2	3.5±0.2	8±2
Maltodextrin	1:1	5.6±0.1	10±1
	1:2	3.3±0.2	12±1

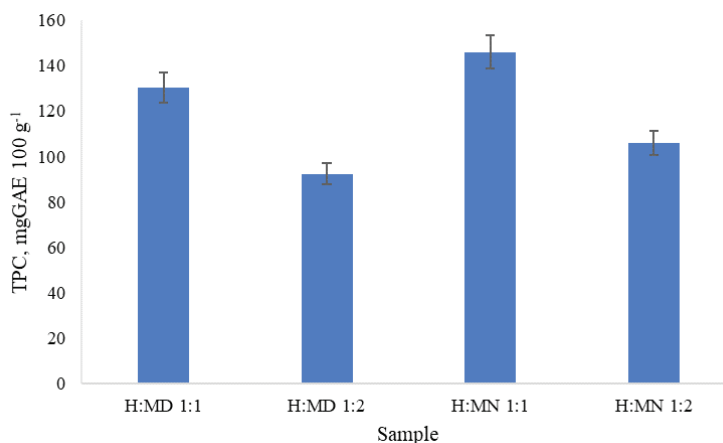


Figure 1. Total phenolic content in honey: maltodextrin 1:1 (H:MD 1:1), honey: maltodextrin 1:2 (H:MD 1:2), honey: mannitol 1:1 (H:MN 1:1), honey: mannitol 1:2 (H:MN 1:2) powder samples.

Results and Discussion

Freeze-drying method was performed to obtain honey powder samples. This dehydration technique is well-known in pharmaceuticals as bioactive compounds preserving drying method (Scutellà *et al.*, 2018). Overall, four formulations were prepared to obtain honey powders. The moisture content in the samples varied from 3.3 to 5.6%. The moisture content in each analysed sample is shown in Table 2.

Maltodextrin and mannitol both are widely used carriers. Maltodextrin is commonly used for drying experiments in food industry (Siccama *et al.*, 2021; Teo *et al.*, 2021). It is usually added during spray drying process to increase the glass transition temperature of drying agent (Hebbar, Rastogi & Subramanian, 2008). Mannitol is a popular non-hygroscopic additive in freeze-drying formulations for pharmaceutical experiments (Kaialy & Nokhodchi, 2015). Mannitol is sugar alcohol, which is used for providing a stable matrix structure during freeze-drying (Mehta *et al.*, 2013).

Total phenolic content in the freeze-dried honey powder samples and fresh buckwheat honey was determined by Folin–Ciocalteu method. The method is based on the reaction between the Folin–Ciocalteu reagent and phenolic compounds, which forms a

coloured complex. Spectrophotometric method was used to measure the absorbance of coloured complex. The measured absorbance data was used to quantify total phenolic content in the samples. The total phenolic content of the samples ranged from 92 to 146 mg GAE 100 g⁻¹ dry matter. High concentrations of phenolics were detected in the samples, where the ratio of honey and carrier was 1:1 (Figure 1). The sample, which contained mannitol, showed the highest content of total phenolics.

Difference in the content of total phenolic compounds in the samples, where the proportion of honey and carrier was 1:2, was not statistically significant ($p > 0.05$). Total phenolic content was analysed in buckwheat honey, which was used for drying experiments. The content of total phenolic in the honey sample was 175 mg GAE 100 g⁻¹ dry matter. Statistically the difference in values between buckwheat honey and obtained dry food products was not significant ($p > 0.05$). Samborska and co-workers (Samborska *et al.*, 2019) also used maltodextrin as carrier in their spray drying experiments to obtain honey powder. In the research, the scientists used different formulations of rapeseed honey and maltodextrin. The highest content of total phenolics was detected in the sample, where honey and maltodextrin ratio was

80:20. In their case, spray drying with dehumidified air was performed to obtain honey powder. The determined value of total phenolics was 78.5 ± 3.7 mg GAE 100 g^{-1} dry matter (Samborska *et al.*, 2019).

Antiradical activity was examined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent, which in reaction with antioxidants forms a yellow solution, which is the colour of the reduced form of DPPH. The absorbance of the coloured solutions were measured and used to determine the antiradical activity of analysed samples. The antiradical activity of the analysed samples were expressed as IC_{50} value. The IC_{50} value shows the ability of sample to inhibit 50% of free radicals. The lower IC_{50} value of the sample is detected, the greater ability it has to neutralize free radicals. The obtained IC_{50} values ranged from 6 to 12 mg mL^{-1} (Table 2). The powders, which contained mannitol, showed the lowest IC_{50} values, and they had a greater ability to neutralize free radicals than the powders, which contained maltodextrin. The reason, why mannitol showed a greater ability to neutralize DPPH, is that mannitol also has antioxidant properties (den Hartog *et al.*, 2010). The IC_{50} value of used buckwheat honey was $3 \pm 2 \text{ mg mL}^{-1}$. Comparing both used carriers and their influence to antiradical activity in powders, maltodextrin showed statistically significant influence to the antiradical activity ($p < 0.05$), but mannitol's influence was not statistically significant ($p > 0.05$).

The freeze-drying process involves very low temperatures and pressure, which allow to protect temperature sensitive phenolic compounds from degradation (Kaialy & Nokhodchi, 2015). As honey is a hygroscopic food product, it is difficult to obtain dehydrated honey without adding carrier materials (Ganaie *et al.*, 2021). Obtaining dehydrated honey samples by this method was not time efficient and

should be modified to optimize drying time and increase the content of honey. Unfortunately, to authors' knowledge there are not scientific publications on obtaining honey powder, where mannitol would be used as a carrier. The experiments showed that mannitol could be used as carrier material for food drying experiments. The application of mannitol as a carrier material in drying experiments might be limited due to its laxative effect. The laxative effect of mannitol might be observed if the daily intake of mannitol exceeds 20 g (Grembecka, 2015). There are many studies (Mooradian *et al.*, 2017; Sahin *et al.*, 2018), where mannitol and other sugar alcohols have been investigated and considered as sucrose substitutes. Honey powder is a relatively new food product, which still needs to be investigated. The further investigations of honey powder would allow to present it to food consumers as an innovative and examined dry food product.

Conclusions

Freeze-drying could be applied as an alternative dehydrating method for obtaining honey-rich powder. Total phenolic content and antiradical activity in the prepared honey powders depend on honey content in the experimental formulations. The obtained powdered samples, which contained honey and mannitol, showed the highest content of total phenolic compounds than the samples, which contained honey and maltodextrin at the same ratio.

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