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### PHYSICOCHEMICAL CHARACTERISTICS AND MELISSOPALYNOLOGICAL ANALYSIS OF COMMERCIAL BUCKWHEAT HONEY FROM POLAND

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#### KEYWORDS

Buckwheat honeys

Honeys type

*Fagopyrum*

Commercial honey

#### ABSTRACT

Buckwheat honey is widely consumed by consumers due to its numerous health-promoting properties. Characteristically it is dark, tea-like in colour, sharp, tickly, and sweet in flavour, and has smelled of buckwheat flowers. In the current study, various commercial honey samples were examined to test the quality of buckwheat honey samples available in the market. The research materials were comprised of 15 samples of honeys from 4 voivodships, among these, 5 samples were collected from the Świętokrzyskie Voivodship, 4 from the Lesser Poland Voivodship, 4 from the Lubelskie Voivodship, and reset 2 samples from the Podkarpackie Voivodship. Melissopalynological analyses of investigated honeys' samples revealed that all samples had at least 45% of *Fagopyrum* pollen content, which means that researched honeys complied with the standards of the International Commission for Bee Botany (ICBB) for buckwheat honey. Honeys' samples had an average water content of 15.3% ( $\sigma= 1.24$ ), and electrical conductivity at  $0.37 \text{ mS}\cdot\text{cm}^{-1}$ . Therefore, all beekeepers correctly marked their honey type as buckwheat honey simply using the organoleptic properties of their honeys and observing their bees collecting pollen and honeydew.

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## 1 Introduction

Buckwheat (*Fagopyrum esculentum* Moench) is one of the most useful plants both for honeybees and other pollinators. This taxon provides insects with large amounts of nectar and pollen throughout the summer and its honey yield ranges from 40 to 494 kilograms from one hectare (Pasini et al., 2013). Buckwheat honeys are some of the most popular types of honey in Eastern Europe, including Poland. Nonetheless, they are also produced in Canada, California, and China (Dalby, 2000; Persano et al., 2004; Pasini et al., 2013).

All varieties of buckwheat honey are specific in their organoleptic characteristics. This honey is dark, tea-like in colour, and turns brown after it has crystallized. Its flavour is sweet, sharp, and tickly. The fragrance is similar to that of buckwheat flowers (Salonen, 2011; Panseri et al., 2013). *Fagopyrum* pollen is frequently found in multiflorous honeys (Pasini et al., 2013). The most common constituents in buckwheat honeys are aldehydes like 3-methylbutanal, 2-methylbutanal and the short-chain carboxylic acid isovaleric acid (3-methylbutyric acid), which may be used as markers for this type of honey (Machado et al., 2020). Buckwheat honeys are very salubrious, and nutrient-rich source for a wealth of health benefits, such as anti-inflammatory and antibacterial properties, natural antioxidants properties, high contents of sugars, proteins, and total phenols, boost in heart health, prevention of certain cancers, and stronger immune system (Schramm et al., 2003; Brudziński et al., 2012; Panseri et al., 2013; Deng et al., 2018). Furthermore, buckwheat honeys exhibit high antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Deng et al., 2018).

Real buckwheat honey has many valuable health properties; therefore this research aimed to check whether the commercial honeys available on the market were well assigned as buckwheat honeys.

## 2 Materials and Methods

The research on honey types was carried out under the laboratory of the Immunobiology, Departments of the Maria Curie-Skłodowska University. The study material was collected from commercial apiaries situated in south-eastern Poland. In total, 15 samples were collected from 4 voivodships, including 5 samples from the Świętokrzyskie Voivodship, 4 samples from the Lesser Poland Voivodship, 4 samples from the Lubelskie Voivodship, and 2 samples from the Podkarpackie Voivodship. All samples were collected in 2017.

Water content in the samples was estimated using the PAL-22S refractometer (Tahoun & Shehata 2015). A small amount of liquid honey was placed on the refractometer's prism and the percentage

content of water was read in turn. The result was expressed in the arithmetic mean from three readings.

The electrical conductivity was measured in the honey solution at the temperature of 20°C using the TDS/EC conductometer. For this,  $M = (5g * 100\%) / MS$  formula was used, where M was the necessary weight (g), MS is the dry mass content, which equaled 100% of dry mass (%) (Živkov et al. 2018). The arithmetic mean from three consecutive readings was used to obtain the final result.

Pollen analysis was performed under the guidelines of the International Commission for Bee Botany (ICBB) (Louveaux et al., 1970). 10g of honey was scooped from every honey sample and 20 ml of distilled water was added in and mix well to dissolve it completely. The resulting suspension was centrifuged twice in the 5804R centrifuge for 10 minutes at 3,000 revolutions per minute. The sediment was suspended in 2 ml of liquid which was left above the sediment with pollen grains. An automatic pipette was used to place 50 µl of properly mixed suspension onto slide glasses. The examination under the Olympus CX21 light microscope was carried out at the magnification that was most suitable for identifying the various elements in the sediment (400 to 1000x). The microscopy analysis was administered using the 600-time engagement. At least 300 grains of pollen grains from nectar-yielding pollen plants were counted. The main types and densities of pollen grains and the relative frequencies of each pollen type were determined according to Panseri et al. (2013). The sum of pollen grains from nectar-yielding pollen was adopted as 100%, and subsequently, the percentage share of each taxon was calculated. On the behalf of observations, four share groups were used and these are predominant pollen types with at least 45% share in the sample (D); secondary pollen types with the share ranging from 16 to 45% in the sample (S); important minor pollen types with the share ranging from 3 to 16% (I); and minor pollen types with the share in the sample not exceeding 3% (R). The share of the *Fagopyrum* pollen needed to amount to at least 45% for the honey to be classified as buckwheat honey.

### 2.1 Statistical Analysis

The data were analyzed using statistical methods and software Statistica (version 12.0, StatSoft Inc., USA) at the significance level of  $\alpha = 0.05$ . The comparisons of quantitative variables in two groups were conducted with either the Student t-test (in the case of normal distribution in both groups) or with Man-Whitney test (otherwise).

## 3 Results and Discussion

Physicochemical characteristics of the buckwheat honey are represented in table 1, these results revealed that the sample from the Świętokrzyskie Voivodship had the lowest percentage content

Table 1 Physicochemical characteristics of the examined honeys

	Sample No.	Participation in the sample (%)			Electrical conductivity (mS · cm <sup>-1</sup> )
		<i>Fagopyrum</i> pollen	water	sugars	
Podkarpackie	1	48.0	17.7	82.3	0.32
	2	46.0	19.1	80.9	0.44
Lesser Poland	1	68.0	16.2	83.8	0.37
	2	45.0	19.0	81.0	0.35
	3	62.2	16.8	83.2	0.39
	4	45.5	18.2	81.8	0.28
Świętokrzyskie	1	68.1	18.2	81.8	0.29
	2	49.0	17.7	82.3	0.33
	3	69.0	16.8	83.2	0.38
	4	50.5	15.3	84.7	0.32
	5	46.0	15.6	84.4	0.44
Lubelskie	1	53.0	15.6	84.4	0.40
	2	60.5	18.0	82.0	0.39
	3	45.0	19.0	81.0	0.43
	4	50.0	16.2	83.8	0.38

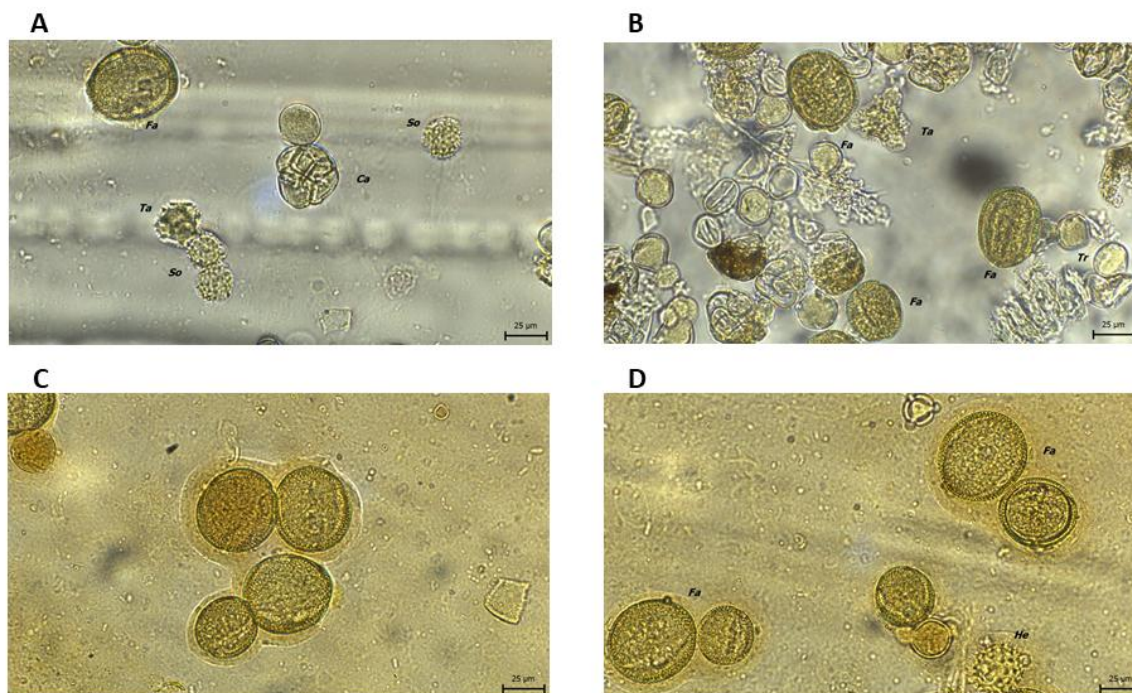


Figure 1 Microscopic images of honey samples with *Fagopyrum* pollen grains. A. The honey sample from Lubelskie Voivodship, B. The honey sample from Podkarpackie Voivodship, C. The honey sample from Lesser Poland Voivodship, D. The honey sample from Świętokrzyskie Voivodship. Abbreviations: *Ca*- *Calluna*, *Fa*- *Fagopyrum*, *He*- *Helianthus* type, *So*- *Solidago* type, *Ta*- *Taraxacum* type, *Tr*- *Trifolium repens*

Table 2 Pollen taxa of nectariferous plants and their contribution in honeys of southeast Poland

Taxa	Nectariferous plants – voivodship and sample No															
	Podkarpackie		Lesser Poland				Świętokrzyskie					Lubelskie				
	1	2	1	2	3	4	1	2	3	4	5	1	2	3	4	
<i>Fagopyrum</i>	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
<i>Phacelia</i>		R	R	S	R	R	S	S	R	R	R	R	R		R	
<i>Solidago</i> type	R	S	R	R	R	R	R	R		R	S	R		S	S	
<i>Salix</i>		R	R	R	R		R	R	R	R	R	R			R	
<i>Trifolium repens</i> .l.		R		R	R		R	R	R	R	R	R		R	R	
<i>Prunus</i> type	R		R	R			R	R	R	R		R	R	R		
<i>Centaurea cyanus</i>		R		R	R	R	R				R	R	R		R	
<i>Achillea</i> type	S	R		R		R			S		R	R		R		
Brassicaceae (other)		R	S	R			R		R		R	R	R			
<i>Helianthus</i> type	R		R		R			R	R	R		R	R			
<i>Taraxacum</i> type		R	R	R	R				R		R			R	R	
<i>Aster</i> type			R			R	R	R		R		I				
<i>Tilia</i>	R					R	R		R			R		R		
<i>Trifolium pratense</i>				R			R		R	R	R					
<i>Rubus</i> type	R					R			R			R				
<i>Brassica napus</i>					R									R	R	
<i>Cirsium</i> type			R			R			R							
<i>Aesculus</i>	R											R				
<i>Anthriscus</i> type			R									R				
<i>Calluna</i>					R							R				
<i>Impatiens</i>				R					R							
<i>Malus</i> type						R			R							
<i>Caryophyllaceae</i>									R							
<i>Convolvulus arvensis</i>										R						
<i>Cucurbita pepo</i>												R				
The number of plant taxa in the sample	Nectariferous plants	8	9	11	12	10	10	11	8	16	10	10	17	6	8	8
	wind-pollinated plants	2	2	1	1	4	1	3	2	3	4	2	1	3	3	4
	Total	10	11	12	13	14	11	14	10	19	14	12	18	9	11	12

Pollen contribution: D – predominant >45%, S – secondary 16-45%, I – important minor 3-15%, R - minor pollen<3%

of water (15.3%), whereas the sample from the Podkarpackie Voivodship had the highest content (19.1%) (Table 1). The average percentage content of water in all samples was 17.4%. Proper electrical conductivity ranged from 0.28 – 0.44 mS\*cm<sup>-1</sup>,

and the average for all samples was  $0.37 \text{ mS}\cdot\text{cm}^{-1}$  (Table 1). All analyzed physicochemical characteristics were following the regulations for honey (EU Council Directive 2014).

The results of melissopalynological analyses are shown as the percentage of each pollen type in table 2. Pollens were classified as predominant (concentration equal to or upper than 45%), secondary (concentration between 16% and 45%), important minor (concentration between 3% and 15%), or minor pollen (concentration less than 3%) (Flores et al. 2015). The share of the *Fagopyrum* pollen in commercial buckwheat honeys ranged from 45.0 to 69.0% (Table 2). Further, one sample included 8 to 16 taxa of nectar-yielding plants and 1 to 4 taxa of non-nectar-yielding plants. Pollen grains of the *Achillea* type, the *Solidago* type, Brassicaceae (others), and *Phacelia* had secondary shares in the samples. Important minor was classified as *Aster* type. Minor pollens were classified to *Phacelia*, *Solidago* type, *Salix*, *Trifolium repens*, *Prunus* type, *Centaurea cyanus*, *Achillea* type, Brassicaceae (other), *Helianthus* type, *Taraxacum* type, *Aster* type, *Tilia*, *Trifolium pratense*, *Rubus* type, *Brassica napus*, *Cirsium* type, *Aesculus*, *Anthriscus* type, *Calluna*, *Impatiens*, *Malus* type, *Caryophyllaceae*, *Convolvulus arvensis*, and *Cucurbita pepo* (Figure 1).

Melissopalynological analyses confirmed beekeepers' declarations and facilitated qualifying all researched samples as buckwheat honey type complying with the International Commission for Bee Botany (ICBB) (Louveaux et al., 1970). The share of the *Fagopyrum* pollen in the investigated honeys' samples was very high (predominant pollen types in a sample and ranged from 45.0% to 69.0%).

Melissopalynological analyses of buckwheat honeys in Poland was investigated since 1966 when Woźna (1966) described buckwheat honeys traded in Poland. The share of *Fagopyrum* pollen in the studied samples was decidedly lower than the obtained in the current study and ranged from 4.7% to 38.2% (Woźna, 1966). On the other hand, Wróblewska (2002) reported the 45.1% share of the *Fagopyrum* pollen in the buckwheat honey from Podlasie. Moreover, this author stressed that *Fagopyrum* pollen had a secondary share in multiflorous honeys from Podlasie. Puścion-Jakubik et al. (2020) also studied buckwheat honeys being traded in Poland, and according to their findings, the share of *Fagopyrum* pollen ranged from 33% to 77%. Having classified three samples as varieties of buckwheat honeys from Lombardy, Panseri et al. (2013) marked the share of *Fagopyrum* pollen at 45.5%, 46.0%, and 52.0% respectively.

Further, the results of current study also revealed that buckwheat honeys have characteristically low water content, and it ranges from 15.3% to 19.1% (Table 1). Generally, information's regarding the availability of water (moisture) content in honeys are

in scanty. Although Majewska et al. (2017) reported 18.6% to 21% water content in honeys, this difference might be due to the difference in the sampling procedure or time of sampling. Similarly, Džugan et al. (2020) also reported 16.2% to 21.0% water content in the collected samples. While Nešović et al. (2020) obtained values below 17.1% for the water content of buckwheat honey samples originated from Serbia and Poland.

Electrical conductivity in buckwheat honeys ranged from 0.28 to  $0.44 \text{ mS}\cdot\text{cm}^{-1}$ , and the average buckwheat conductivity was at  $0.37 \text{ mS}\cdot\text{cm}^{-1}$  ( $\sigma = 0.05$ ) (Table 1). These results are in agreement with the findings of Nešović et al. (2020), those who reported 0.32 and  $0.43 \text{ mS cm}^{-1}$  values in the buckwheat honey samples collected from the Serbian region, while for the Polish honey electrical conductivity was reported  $0.21 \text{ mS cm}^{-1}$  and  $0.28 \text{ mS cm}^{-1}$  which was the lower than the current study. The results of Majewska et al. (2017) are in agreement with the findings of the current study, these researchers reported proper electrical conductivity from 0.11 to  $0.49 \text{ mS}\cdot\text{cm}^{-1}$ .

Some other studies showed that in most of the cases the classification of honeys made by their producers was incorrect and when the research honey samples were analyzed and discussed with consideration of the determined pollen concentrations and conductivity, the real honey type was different than the type notified by the manufacturer (Halagarda et al., 2020). However, the current study suggests that the commercial buckwheat honeys had at least 45% of *Fagopyrum* pollen content, in this manner current results complied with the standards of the International Commission for Bee Botany (ICBB) (Louveaux et al., 1970). Buckwheat honey samples had an average water content of 15.3% ( $\sigma = 1.24$ ), and electrical conductivity at  $0.37 \text{ mS}\cdot\text{cm}^{-1}$ .

## Conclusion

Melissopalynological analyses confirmed beekeepers' declarations and facilitated qualifying all researched samples as buckwheat honey type complying with the International Commission for Bee Botany (ICBB). All beekeepers correctly marked their honey types simply using organoleptic properties of their honeys and observing their bees collecting pollen and honeydew. Conceivably, beekeepers are becoming more aware of the principles of determining the variety of honey. Apparently, buckwheat honey is honey with very distinctive features that allow for its easy identification even without honey melissopalynological analyses and conductivity.

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### Conflict of interest

The authors declare that there is no conflict of interest.

### References

- Brudzynski K, Abubaker K, Wang T (2012) Powerful bacterial killing by buckwheat honeys is concentration-dependent, involves complete DNA degradation and requires hydrogen peroxide. *Frontiers in Microbiology* 3: 242.
- Dalby R (2000) Classic American honey plant: buckwheat. *American Bee Journal* 140: 485–486.
- Deng J, Liu R, Lu Q, Hao P, Xu A, Zhang J, Tan J (2018) Biochemical properties, antibacterial and cellular antioxidant activities of buckwheat honey in comparison to manuka honey. *Food Chemistry* 252:243-249 doi: 10.1016/j.foodchem.2018.01.115.
- Dżugan M, Grabek-Lejko D, Swacha S, Tomczyk M, Bednarska S, Kapusta I (2020) Physicochemical quality parameters, antibacterial properties and cellular antioxidant activity of Polish buckwheat honey. *Food Bioscience* 34: 100538. DOI: <https://doi.org/10.1016/j.fbio.2020.100538>.
- EU Council Directive (2014) 2001/110/EC of 20 December 2001 relating to honey. 2002 OJEC. L10, 47-51. Available at <https://eur-lex.europa.eu/eli/dir/2001/110/2014-06-23> access on 21st December 2020.
- Flores MSR, Escuredo O, Seijo MC (2015) Assessment of physicochemical and antioxidant characteristics of *Quercus pyrenaica* honeydew honeys. *Food Chemistry* 166: 101–106.
- Halagarda M, Groth S, Popek S, Rohn S, Pedan V (2020) Antioxidant Activity and Phenolic Profile of Selected Organic and Conventional Honeys from Poland. *Antioxidants (Basel)*. 9(1): 44. <https://doi.org/10.3390/antiox9010044>.
- Louveaux J, Maurizio A, Vorwohl G (1970) Methods of melissopalynology (republished and updated). *Bee World* 59: 139–157.
- Machado AM, Miguel MG, Vilas-Boas M, Figueiredo AC (2020) Honey Volatiles as a Fingerprint for Botanical Origin—A Review on their Occurrence on Monofloral Honeys. *Molecules* 25: 374. DOI: <https://doi.org/10.3390/molecules25020374>.
- Majewska E, Drużyńska B, Kowalska J, Wołosiak R, Ciecierska M, Derewiaka D (2017) Zastosowanie metod fizykochemicznych i chemometrycznych do oceny jakości i autentyczności botanicznej miodów gryczanych. *Zeszyty Problemowe Postępów Nauk Rolniczych* 589: 59-68.
- Nešović M, Gašić U, Tosti T, Horvacki N, Šikoparija B, Nedić N, Blagojević S, Ignjatović L, Tešić Ž (2020) Polyphenol profile of buckwheat honey, nectar and pollen. *Royal Society Open Science* 7: 201576. <https://doi.org/10.1098/rsos.201576>.
- Panseri S, Manzo A, Chiesa LC, Giorgi A (2013) Melissopalynological and Volatile Compounds Analysis of Buckwheat Honey from Different Geographical Origins and Their Role in Botanical Determination. *Journal of Chemistry* <https://doi.org/10.1155/2013/904202>.
- Pasini F, Gardini S, Marcazzan S, Caboni M (2013) Buckwheat honeys: Screening of composition and properties. *Food Chemistry* 141: 2802-2811.
- Persano OL, Piana L, Bogdanov S, Bentabol A, Gotsiou P, Kerkvliet J, Martin P, Morlot M, Ortiz-Valbuena A, Ruoff K, Von der Ohe K (2004) Botanical species giving unifloral honey in Europe. *Apidologie* 35: 82-93.
- Puścion-Jakubik A, Socha K, Borawska MH (2020) Comparative study of labelled bee honey from Poland and the result of the melissopalynological analysis, *Journal of Apicultural Research* 59 (5): 928-938. DOI: 10.1080/00218839.2020.1726035.
- Salonen A (2011) Boreal unifloral honeys: Screening of composition and properties. Publications of the University of Eastern Finland. Dissertations of University of Eastern Finland in Forestry and Natural Sciences, downloaded from [http://epublications.uef.fi/pub/urn\\_isbn\\_978-952-61-0589-5/urn\\_isbn\\_978-952-61-0589-5.pdf](http://epublications.uef.fi/pub/urn_isbn_978-952-61-0589-5/urn_isbn_978-952-61-0589-5.pdf).
- Schramm DD, Karim M, Schrader HR, Holt RR, Cardetti M, Keen CL (2003) Honey with High Levels of Antioxidants Can Provide Protection to Healthy Human Subjects. *Journal of Agricultural and Food Chemistry* 51: 1732-1735. doi: 10.1021/jf025928k.
- Tahoun IF, Shehata AB (2016) Preparation of Honey Reference Material for Water Content by Karl Fisher and Refractometric Methods. *MAPAN* 31: 25–29 doi:10.1007/s12647-015-0156-y.
- Woźna J (1966) Obraz pyłkowy i barwa niektórych odmianowych miodów handlowych. *Pszczelnicze Zeszyty Naukowe* 10: 1-4.
- Wróblewska A (2002) Rośliny pożytkowe Podlasia w świetle analizy pyłkowej produktów pszczelich. *Pracahabilitacyjna Wydawnictwo Akademii Rolniczej w Lublinie*.
- Živkov BM, Popov N, Vidaković S, Ljubojević Pelić D, Pelić M, Mihaljev Željko, Jakšić S (2018) Electrical conductivity and acidity of honey. *Archives of Veterinary Medicine* 11: 91-101.