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Physicochemical characteristics of bee pollen collected from Virovitica-Podravina County

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ABSTRACT

Pollen grains are located on the anthers of the flowers, bees collect them and mix them with secretions from their salivary glands and nectar and carry them to the hives in the baskets situated on their hind legs. According to the chemical composition, bee pollen is a mixture of proteins, fats, carbohydrates, vitamins and other useful substances for the development of bees in the hive. Due to its nutritional value and potential healthful properties, it is valuable product for beekeeper's income. The aim of this research was to evaluate the physicochemical characteristics of bee pollen in relation to the period of collection and geographical origin from different localities in Virovitica-Podravina County. Eleven bee pollen samples were collected from stationary apiaries in the period of April and May 2022. Palynological analysis showed that dominated pollen in five samples was pollen from *Brassica* sp.; *Malus* spp. and *Salix* spp. pollen dominate in two bee pollen samples while two samples had dominating pollen coming from *Asteraceae* family. Physicochemical analysis showed that pollen collected in the period of April had a lower proportion of ash, total fat and phenols, but a higher proportion of moisture content, proteins and carbohydrates.

Introduction

Honeybees carry flower pollen to the hives where it becomes food for the colony, rich in fats, minerals, and proteins. Bee pollen is a flower pollen, which is also a natural mixture of bee secretions, honey, enzymes, wax and flower pollen. It is stored in the beehives and used as a food source for the whole colony (Ilie et al., 2022). To date, about 250 various chemical compounds have been determined in it (Kieliszek et al., 2018), including carbohydrates, fats, proteins, vitamins, macro- and microelements, antibiotics (inhibins), hormones, enzymes, organic acids, essential oils, polyphenol and others and has attracted increasing attention as a functional food (Lu et al., 2022). Its value lies in proteins containing essential amino acids, lipids, minerals and water- and oil-soluble vitamins and polyphenols (Ares et al., 2018). Bees use it as food for young bees in the hive, but its nutritional value was recognised by the humans centuries ago and it has been used as food and medicine (Aličić et al., 2020). The chemical composition of bee pollen depends strongly on the plant source and geographic origin, together with other factors, such as climatic conditions, soil type, and bee species and activities (Jannesar et al., 2017). Major bee pollen components are carbohydrates (13 - 55%), proteins (10 - 40%) and lipids (1 - 10%) (Bogdanov, 2016). Bioactive compounds comprise approximately 70% of bee pollen (Aličić et al., 2020), giving it antioxidative, anti-inflammatory, antimicrobial, anticancerogenic and immunostimulant properties (Mărgăoan et al., 2019). The colour of the pollen ranges from light yellow to black (Węglińska et al., 2020). Pollen grains, depending of the plant species, differ in shape, colour, size, and weight. The grain shapes are diverse: round, cylindrical, bell-shaped, triangular, or thorny. Their weight is equal to a dozen or several dozens of micrograms. The majority of pollens consists of single grains which are sometimes

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joined with two or more grains (Shubharani et al., 2013). Bee pollen is classified into two groups according to its flower source: monofloral (the major species need to be not less than 80%) and multifloral (which contains pollen from more than one plant taxon) (Gercek et al., 2022). All plants produce a large number of phenolic substances as secondary metabolites in their metabolism (Khoddami et al., 2013). Bee pollen is a good source of polyphenol and flavonoid compounds, which have the ability to scavenge free radicals (Denisow et al., 2016). Bee pollen contains flavonoids such as rutin, quercitrin, isoquercitrin, naringenin, kaempferol, and luteolin. Flavonoids that have antiviral, antibacterial, anti-oxidation, anti-aging, anti-inflammatory, anti-tumor and analgesic activities are among the main bioactive substances in bee pollen and important indicators of the quality of bee pollen (Duan et al., 2019). The most important bioactive components of bee pollen are polyphenols, especially phenolic acids and flavonoids, but also carotenoids (Velásquez et al., 2017), which are responsible for the anti-inflammatory, antimicrobial, antioxidative, anticancerogenic, and immunostimulant properties of bee pollen (Mărgăoan et al., 2019). Such properties have led to the utilization of bee pollen as a valuable dietary supplement and with a great potential for being used as a medicine (Bakchiche et al., 2020). The aim of this research was to determine the characteristics of bee pollen from different localities and different samples collection time from the area of Virovitica-Podravina County. Also, it will be examined weather and in what extent conditions and botany around apiaries have influence on bee pollen composition.

Materials and methods

Sampling of Bee Pollen

Eleven samples of bee pollen were collected at 9 locations in Virovitica-Podravina County, Republic of Croatia: Rezovačke Krčevine, Turnašica, Dugo Selo Lukačko, Brezovljani, Slatinski Drenovci, Đuričići, Sekulinci, Macute and Voćin in the period April/May 2022. All apiaries were stationary. Samples of bee pollen were collected from outdoor and indoor pollen collectors in healthy and strong *Apis mellifera* bee colonies in the amount of 250 grams, depending on weather conditions. The collected bee pollen was kept in well-closed containers in a freezer at -18 °C to prevent the development of moulds and bacteria and to preserve their biological and chemical properties. All analyses were performed in duplicate. During collection, parameters of air temperature (°C) and relative air humidity (%) were measured on a Trotec PC220 pollen grain counter device (Trotec GmbH + Co.KG, Germany).

Determination of Botanical Origin – Melissopalynological Analysis

Microscopic slides of bee pollen samples were prepared according to modified method proposed by Barth et al. (2010). Two grams of fresh bee pollen sample was weighed in the centrifuge tube with addition of 10 mL of 70% ethanol. Mixture was vortexed and placed in ultrasonic bath (Bandelin, Sonorex, Super RK 100 H, Germany) for five minutes. Afterwards the tubes were centrifuged (Sigma 2-16, Sigma Laborzentrifugen GmbH, Germany) for three minutes at 1500 rpm. Ethanol resuspended sediment was subjected to the same procedure again. Sediment was resuspended three times with water (7 mL) and glycerol solution (7 mL, 1:1) and sediment was stirred with Pasteur pipette at the end and spread on the microscopic slide. Melissopalynological analysis was conducted on Optika B-800 microscope (Ponteranica, Italy), where at least 500 pollen grains were counted and identified in each microscopic slide. For the botanical origin identification, CMS Celle's Melyssopalyogical Collection (von der Ohe, 2003) was used.

Moisture Content (%)

Three grams of fresh pollen was weighed in a pre-dried, desiccator cooled aluminum container with a lid. Drying was conducted in vacuum dryer (Christ, Alpha LSC plus, Germany) under the following conditions: temperature 60 °C, pressure 6.7 kPa (<50 mmHg). After appropriate drying time, the containers were covered, cooled for a minimum of 45 minutes in a desiccator and weighed. The procedure was repeated until the constant weight was reached.

pH value

pH value was measured in an aqueous solution, which was prepared by mixing ten grams of fresh pollen sample in 75 mL of distilled water and measured with a digital pH meter Fiveeasy F20 KIT (Mettler Toledo,

Germany). Analyses were conducted in duplicate. Acidity analyses were carried out according to Anjos et al. (2019).

Ash Content (%)

The ash content was determined gravimetrically, according to the AOAC 923.03. In total, three grams of the fresh pollen sample was placed in an annealed porcelain crucible (550 °C), cooled in a desiccator and weighed, and distributed evenly over the crucible. Using an electric heater, the sample was slowly heated until it carbonized. When the smoke stopped developing, the crucible was transferred to a muffle furnace (Nabertherm, Germany) and preheated to 550 °C until ash was obtained. If the residue in the crucible contained unburned carbon particles, it was moistened with a small amount of distilled water and 2 mL of concentrated 65% HNO_3 (LabExpert, Slovenia) after cooling. The crucible covered with a watch glass was heated on an electric heater. Afterwards, the content was dried and burned again in the muffle furnace. Upon completion of incineration, the crucible was placed in a desiccator to cool and weighed. Incineration was repeated until a constant mass. Analyses were conducted in duplicate and results reported to dry weight.

Protein Content (%)

Protein content was determined by Kjeldahl's method where 0.8 g of fresh pollen sample was weighed in a cellophane bract and the procedure of wet digestion with addition of catalizator ($CuSO_4 \cdot 5H_2O$, Gram-Mol, Croatia), salt (Na_2SO_4, Gram-Mol, Croatia) and sulphuric acid (95-97% purity, LabExpert, Slovenia) was conducted on Behrotest InKjel M digestion unit (Behr Labor-Technik, Germany). Distillation was conducted on Behrotest Behr S2 automatic steam distillation apparatus (Behr Labor-Technik, Germany) with addition of 35% NaOH solution (Gram-Mol, Croatia). The distillate containing NH_4OH was introduced into the Erlenmayer flask which contains the acid of known molarity (0.1 M HCl). At the end of the distillation excess acid was titrated with 0.1 M NaOH (Gram-Mol, Croatia). Nitrogen content was calculated as follows:

$$\% \, \mathrm{N} = \frac{(\mathrm{a} - \mathrm{b}) \cdot f \cdot 1.4}{\mathrm{c} \cdot 10} \qquad (1)$$

where:

a – 0.1 M NaOH solution used for titration of blank (mL);
b – 0.1 M NaOH solution used for titration of sample (mL);
f – factor of used 0.1 M NaOH solution;
c – mass of the sample (g).

Protein content was calculated with multiplying nitrogen content calculated according to formula (1) with factor 5.6. Analyses were conducted in duplicate and results reported to dry weight.

Total Fat Content (%)

The total fat content was determined gravimetrically, according to the AOAC 963.15. In total, three grams of the fresh pollen sample was weighed and added into a 250 mL beaker, with a few boiling beads, 45 mL of boiling water and 55 mL of 8 M HCl (Gram-Mol, Croatia). The baker was covered with a watch glass, heated on an electric heater, and 15 minutes was measured after the start of the boiling. After the cooling, the watch glass was washed with distilled water and the content was filtered through filter paper. The beaker was rinsed several times with distilled water, and the content of the filter paper was rinsed with distilled water until there was a negative reaction to the chlorides. The reaction was tested with 0.1 M $AgNO_3$ (Kefo d.o.o., Slovenia). After digestion, the filter paper was transferred to a test tube, placed in a glass and dried for 2 h at 100 °C in dryer UF110 (Memmert GmbH, Germany). After the drying, the watch glass, which the extraction thimble was located on during drying, was wiped with cotton wool soaked in petroleum ether (Carlo Erba, France) and the cotton wool was placed in the extraction thimble.

This was followed by fats extraction, according to Soxhlet (Behr Labor-Technik, Germany). A Soxhlet flask with several boiling beads was dried in an oven at 100 °C (± 2 °C) for one hour, cooled in a desiccator for 30 minutes and weighed on an analytical balance. The dried extraction thimble was placed in a Soxhlet apparatus extractor which was connected with the flask. For extraction petroleum ether (Carlo Erba, France) was used.

The extraction lasted 4 hours, i.e., the extractor was emptied at least 30 times. The solvent was distilled off, the residue was evaporated on a water bath and the

flask was dried in an oven at 100 °C (± 2 °C). The flask was cooled in a desiccator for 30 min, weighed on an analytical balance and dried again for 30 minutes at a temperature of 100 °C (± 2 °C) to the constant weight. Analyses were conducted in duplicate and result reported to dry weight.
The total fat content (%) was calculated from mass of extracted lipid (g) × 100/sample weight (g).

Determination of Carbohydrate Content by HPLC Method

Analysis of carbohydrates in bee pollen samples was performed on a high-pressure liquid chromatography (HPLC) (Shimadzu Corp., Japan) with a refractive index detector (RID). Identification and quantification of glucose, fructose, sucrose, maltose, melezitose and raffinose was carried out. An Agilent Zorbax NH_2 column, 4.6×250 mm in size, filled with 5 µm particles, at a column temperature of 30 °C was used for carbohydrate separation. The mobile phase consisted of acetonitrile (J. T. Baker, Poland) and ultrapurified water in a ratio of 75:25 (v/v), the flow rate of the mobile phase was 1.3 mL/min, and the injection volume was 10 µL. For carbohydrate analysis, 1 g of sample was weighed into a 10 mL volumetric flask and 5 mL of methanol: water solution (3:1, v/v) was added, along with 0.1 mL of Carezz I and Carezz II solution each, and the volumetric flask was filled up to labels with a methanol: water solution. Solution was ultrasonicated for 5-10 min, after which it was filtered through the filter paper, and then also through the membrane filter for aqueous solutions with a pore size of 0.22 µm. The filtered solution was placed in an autosampler and analyzed on an HPLC device (Liolios et al., 2018.). Identification and quantification were performed using the computer programme LabSolution Lite (Version 5.52). Quantification was performed using the external calibration method. Analyses were conducted in duplicate and result reported to dry weight.

Total Phenolic Content (mgGAE/g)

Folin-Ciocalteu method (FC) was used for determination of total phenolic content according to the Singleton et al. (1999) expressed as mg of gallic acid per gram of bee pollen. Distilled water (6 mL) and FC reagent (0.5 mL) (Reagecon, Ireland) were added to 100 µL of diluted bee pollen extract (0.1 mL extract + 0.1 mL distilled water). Solution of 20% Na_2CO_3 (1.5 mL) (Panreac, Spain) was added in the sixth minute and 10 mL volumetric flask was filled with distilled water to the mark and left to stand in dark for two hours. Calibration curve was made with testing solutions of gallic acid (98% purity, Sigma-Aldrich, Switzerland) in concentrations from 0.1 to 1.0 mg/mL and absorbance was measured at 760 nm (UV-Vis spectrophotometer uniSPEC; LLG Labware, Germany). Analyses were conducted in duplicate and result reported as mg of gallic acid per gram of bee pollen.

Data Analysis

Mean values, minimum, maximum and standard deviations were calculated for each parameter using Microsoft Excel 2010 (Microsoft Corp.) software.

Results and discussion

The determined samples were collected in the period from April to May (Table 1). Six apiaries are stationed in an orchard next to family houses where there is also a forest nearby, while five samples are stationed right in the forest area (Table 1).

Determination of Botanical Origin – Melissopalynological Analysis

Two samples are monofloral, because they contain more than 80% of one type of pollen grains (Campos et al., 2008), sample S4 contains 88.6% *Brassica* sp. and sample S5 contains 89.7% *Brassica* sp., while the other samples are multifloral. A total of 25 types of pollen grains were identified in all samples (Table 2).

Characterization of Bee Pollen by Physicochemical Analyses

The chemical composition of bee pollen shows variations between samples in both multifloral and monofloral samples, which may be the cause of different botanical and geographical origin, types of plants surrounding apiaries and storage conditions (Nogueira et al., 2012).
The pH value in this study ranged from 4.74-5.83, while the mean value was 5.20. In the research of Thakur et al. (2018) the pH results were in the same range (4.74-5.48) (Table 3).

Table 1. Pollen collection conditions

Sample	Number of hives	Month of harvest	Pollen colector	Location of the apiary	Air temperature (°C)	Relative air humidity (%)	Weather conditions
S1	30	april	external	orchard	10.1	74.8	cloudy
S2	30	april	internal	orchard	10.0	74.3	cloudy
S3	4	april	internal	forest	19.3	42.6	sunny
S4	6	april	internal	orchard	21.8	38.8	sunny
S5	5	may	external	orchard	16.5	62.4	cloudy
S6	8	may	internal	orchard	19.9	55.8	cloudy
S7	12	may	external	forest	19.8	60.1	cloudy
S8	8	may	external	forest	18.7	60.3	cloudy
S9	11	may	external	forest	19.8	58.8	cloudy
S10	24	may	external	orchard	21.4	54.8	partly cloudy
S11	4	may	internal	forest	21.9	49.3	sunny

Table 2. Pollen spectrum of collected monofloral and multifloral bee pollen samples

	Samples (%)										
Species	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**
Acer spp.						5.2	4.1		4.0		
Aesculus hippocastanum	2.3					3.1					
Apiaceae			8.6			6.7	3.1	2.3	4.1		
Asteraceae	3.7	3.1	5.9			7.3	2.9	1.7	3.2		6.8
Asteraceae (Taraxacum form)						2.8				3.7	26.3
Betula pendula	3.9					6.2			4.6		
Brassica sp.	45.4	54.0	17.5	88.6	89.7	12.3	21.4	15.3	15.1	6.2	12.6
Campanula spp.	1.8										
Cornus sanguinea					6.1	3.6	4.0	2.0	5.4	7.2	7.5
Lamiaceae	2.8										
Malus spp.							16.8	29.3	31.2	33.2	16.0
Poaceae					3.1					3.2	
Prunus spp.			4.1			7.1		2.8	3.2		3.3
Quercus spp.								8.7	11.1		3.5
Robinia pseudoacacia						5.6	15.0	5.0	7.9	3.0	2.7
Rosaceae	6.3							5.6			
Rosaceae (Prunus form)	5.2	7.7	11.6			6.1	7.1				
Rosaceae (Rubus form)	6.8	6.0	8.0			6.1					
Rubus spp.			2.4			3.2	14.6	14.6	3.8	4.5	6.2
Salix spp.	11.3	23.6	13.6	7.8	1.1	15.8	11.2		6.5	39.2	9.7
Taraxacum officinale	4.0	2.4	22.1	3.6							
Trifolium pratense		3.1	6.3			2.8		7.3			3.3
Trifolium repens						3.2		5.7			2.3
Trifolium spp.	6.5										
Verbascum nigrum						3.1					

Table 3. Physicochemical characteristics of bee pollen samples

Sample	pH value	Moisture (%)	Ash (%) [*1]	Total fat (%) [*1]	Protein (%) [*1]	Total phenols (mg GAE/g) [*1]
S1	5.15	20.15 ± 0.17	2.83 ± 0.00	8.98 ± 0.27	23.04 ± 0.54	9.71 ± 0.07
S2	5.00	20.64 ± 0.18	2.77 ± 0.00	10.47 ± 0.27	23.68 ± 0.45	9.65 ± 0.05
S3	5.59	14.83 ± 0.05	2.66 ± 0.06	10.97 ± 0.20	21.00 ± 0.05	8.00 ± 0.01
S4	4.87	21.77 ± 0.08	2.81 ± 0.06	10.35 ± 0.05	24.84 ± 0.31	10.33 ± 0.02
S5	4.87	16.31 ± 0.10	2.69 ± 0.03	9.83 ± 0.05	22.92 ± 0.24	9.27 ± 0.02
S6	5.50	19.21 ± 0.02	2.83 ± 0.03	9.39 ± 0.16	23.95 ± 0.44	9.72 ± 0.01
S7	5.39	17.81 ± 0.10	3.50 ± 0.03	10.15 ± 0.20	22.48 ± 0.86	10.35 ± 0.01
S8	5.10	19.70 ± 0.04	3.52 ± 0.00	10.73 ± 0.20	21.03 ± 0.04	10.51 ± 0.04
S9	4.74	19.20 ± 0.08	2.74 ± 0.03	9.75 ± 0.14	22.87 ± 0.68	10.68 ± 0.02
S10	5.19	18.08 ± 0.15	2.89 ± 0.06	9.47 ± 0.16	23.76 ± 0.06	10.75 ± 0.03
S11	5.83	12.47 ± 0.08	2.53 ± 0.02	11.20 ± 0.07	20.79 ± 0.36	9.31 ± 0.01
Mean	**5.20**	**18.19**	**2.89**	**10.12**	**22.76**	**9.84**
SD[2]	**0.01**	**0.09**	**0.03**	**0.16**	**0.37**	**0.03**
Min[3]	**4.74**	**12.47**	**2.53**	**8.98**	**20.79**	**8.00**
Max[4]	**5.83**	**21.77**	**3.52**	**11.20**	**24.84**	**10.75**

*[*1]Results reported to dry weight.*
[2]SD = standard deviation.
[3]Min = minimum value.
[4]Max = maximum value.

Table 4. Carbohydrate content in bee pollen samples

Sample	Fructose (%) [*1]	Glucose (%) [*1]	Sucrose (%) [*1]	Maltose (%) [*1]	Melecitose (%) [*1]	Raffinose (%) [*1]
S1	14.92 ± 0.12	11.70 ± 0.16	8.75 ± 0.01	2.16 ± 0.04	0.41 ± 0.03	ND[5]
S2	15.64 ± 0.18	12.95 ± 0.12	8.05 ± 0.33	1.84 ± 0.27	0.30 ± 0.13	ND
S3	15.93 ± 0.15	14.38 ± 0.23	9.23 ± 0.30	3.77 ± 0.06	0.70 ± 0.06	ND
S4	15.26 ± 0.05	12.20 ± 0.24	3.50 ± 0.11	1.15 ± 0.00	ND	ND
S5	14.77 ± 0.06	12.87 ± 0.10	6.69 ± 0.18	1.32 ± 0.23	ND	ND
S6	11.44 ± 0.46	7.69 ± 0.35	17.70 ± 0.47	2.39 ± 0.02	ND	ND
S7	15.34 ± 1.00	10.23 ± 0.71	11.70 ± 0.76	3.04 ± 0.31	ND	ND
S8	16.94 ± 0.48	11.94 ± 0.16	8.60 ± 0.01	2.14 ± 0.05	ND	ND
S9	17.33 ± 0.12	12.90 ± 0.08	5.40 ± 0.07	2.80 ± 0.09	ND	ND
S10	15.12 ± 0.10	12.47 ± 0.06	11.72 ± 0.10	2.27 ± 0.19	ND	ND
S11	11.92 ± 0.61	10.96 ± 0.56	16.72 ± 0.25	3.15 ± 0.24	ND	ND
Mean	**14.96**	**11.84**	**9.82**	**2.37**	**0.47**	**ND**
SD[2]	**0.30**	**0.25**	**0.24**	**0.14**	**0.07**	**ND**
Min[3]	**11.44**	**7.69**	**3.50**	**1.15**	**0.41**	**ND**
Max[4]	**17.33**	**14.38**	**17.70**	**3.77**	**0.70**	**ND**

*[*1] Results reported to dry weight.*
[2]SD = standard deviation.
[3]Min = minimum value.
[4] Max = maximum value.
[5]ND = not detected.

Samples of fresh bee pollen collected in April had a higher amount of moisture, protein, fructose and glucose, but a lower amount of ash, total fat, total phenol, sucrose and maltose than the samples collected in May. The higher amount of melecitose was detected in the samples S1 and S3, while in all samples raffinose was not detected, regardless of the time of collection. With the arrival of warmer and sunnier days towards the month of May, the amount of moisture decreased, while the amount of ash, total fat, total phenol, sucrose and maltose increased. As expected, *Brassica* sp. was found in all samples by melissopalynological analysis, which indicates that, regardless of whether the apiary was surrounded by a forest or in an orchard, a greater amount of rapeseed pollen was found.

Fresh bee pollen can contain from 10-30% moisture (Thakur et al., 2020; Spulber et al., 2018), which corresponds to the data obtained in this study, where the moisture content was from 12.47-21.77% and the mean value was 18.19% (Table 3). The most similar results to those obtained in this study are the results of Thakur et al. (2018) and Prđun et al. (2021), while the results obtained by Sagona et al. (2017) were slightly higher. With S1 and S2, it can be assumed that the reason for the higher moisture content is due to the fact that they were collected at low air temperature, high relative air humidity, while S4 deviates from the above assumptions, which may be connected with sampling method.

The content of ash - can be influenced by climate, botanical and geographical origin, where the type of soil also has an influence on the mineral content of bee pollen (Yang et al., 2013). Fresh bee pollen contains from 0.5-6.5% ash expressed on the dry weight (Isopescu et al., 2020; Kostić et al., 2015; Khider et al., 2013; Mărgăoan et al., 2012), while the results obtained from this study were in the range of 2.53 – 3.52% expressed on the dry weight (Table 3). Spulber et al. (2018) obtained lower ash content values (1.34-2.81 g/100 g) than those obtained in this study while Thakur et al. (2018) obtained ash content results of 2.27-3.45%, which is very similar to results in this study, unlike the results obtained by Gabriele et al. (2015) where the ash content was 2.55-2.85%.

The total fat content in this study was from 8.98-11.20% expressed on the dry weight, while the mean value was 10.12% (Table 3). These results were in the higher proportion compared to the results of Isik et al. (2019) whose total fat content is 5.50 g/100 g, and quite similar to the results of Thakur et al. (2018) and unlike Liolios et al. (2019), where the proportion of total fat was from 1.15-13.60%. Spulber et al. (2018) obtained lower values of total fat in his study as well as Sagona et al. (2017) in contrast to the data obtained in this study.

The content of proteins is from 20.79-24.84%, expressed on the dry weight with a mean value of 22.76% (Table 3), while the share of -proteins in Isik et al. (2019) is from 30.36 g/100 g. The data obtained in this study are consistent with the results of Spulber et al. (2018), while Thakur et al. (2018) obtained total protein results almost identical to our results, together with Sagona et al. (2017).

The content of total phenols is from 8.00-10.75 mg GAE/g, while the mean value is 9.84 mg GAE/g (Table 3), where Mayda et al. (2020) obtained more content of total phenols in the range of 8.26-43.42 mg GAE/g.

Monofloral bee pollen in sample S4 had the highest proportion of proteins than other multifloral samples. The protein content ranged from 24.84% in sample S4 (monofloral sample) to 20.79% in other multifloral samples (Table 3). In sample S4, which contains 88.6% rapeseed pollen, the moisture content is 21.77%, the ash content is 2.81%, the total fat content is 10.35%, the protein content is 24.84% and the total phenol content is 10.33 mg GAE /g. Straumita et al. (2022) reported that monofloral sample of rapeseed contains 7.32% of moisture, pH was 5.02 and the content of total phenols was 29.94 mg GAE/g per dry weight. Bertoncelj et al. (2018) analyzed a monofloral bee pollen sample containing 87% *Brassicaceae*, where the moisture content was 14.8%, glucose content 13.79 g/100 g, fructose content 13.65 g/100 g, maltose content 0.536 g/100 g, sucrose content 0.07 g/100 g (all expressed on dry weight), while melecitose was not detected.

Carbohydrate content showed significant variations between pollen samples in the range of 24.19-53.88%, while the mean value was 39.04%. Bertoncelj et al. (2018) obtained higher values of glucose, fructose and maltose, but lower proportions of sucrose in contrast to Mărgăoan et al. (2012), where the proportions of glucose and fructose were in the obtained range, but sucrose and maltose were not detected. Liolios et al. (2018) obtained results of a higher proportion of glucose and fructose and a lower proportion of sucrose 0.10-8.24%, while maltose was in the range of data obtained from this study (Table 4). Table 4 shows a significant variation in the proportion of sucrose in all samples. The proportion of sucrose in fresh bee pollen is affected with the botanical origin of the pollen.

Conclusions

The results of this research showed great variability in the physicochemical composition of bee pollen, which depends on the geographical and botanical origin of

the plant species, but also on the location of the stationary apiaries. Sample S6 identified the highest number of plant species. *Brassica* sp. was found in all samples, while *Salix* spp. was found in ten bee pollen samples. Results were as expected, because at the time of the collection of bee pollen it was the flowering season of oilseed rape. For future research, more pollen samples should be taken from higher number of different locations and periods of collection to make a correlation among botanical origin, antioxidant activity and protein content depending on the botanical origin of the plants present around the stationary apiaries. It will be interesting to determine the proportion of heavy metals in bee pollen in relation to the locality and the period of collection, considering the distance of available field crops in certain periods of collection.

Author Contributions: Nela Drača – sampling bee pollen at apiaries; determination of pH value, ash content, total fat content, total phenolic content; wrote the article.

Blanka Bilić Rajs – determination of melissopalynological analysis, moisture content, protein content, carbohydrate content by HPLC method, reviewed the article.

Ivana Flanjak – reviewed the article, supervised the experiment.

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