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Nutritional composition of *Phacelia tanacetifolia* Benth. bee pollen and inflorescences

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The interest in natural products, namely, bee products is actual nowadays. The Bee pollen is a rich source of nutrients and biologically active compounds with numerous biological activities such as antioxidant, antimicrobial, anticancer, anti-inflammatory, etc. The search for new plant raw as a source of natural products has a practical meaning. The goal of this study was to evaluate the biochemical composition of bee pollen and inflorescences of *Phacelia tanacetifolia* Benth. Grown in Slovakia. The plant raw material was collected from the experimental plots of Slovak Agricultural University in Nitra. There were conducted following biochemical analyses: dry matter, protein, ash, lipid, β-carotene, fatty acid, amino acid, and saccharide content. *Ph. tanacetifolia* bee pollen had 73.3% of dry matter, 27.44% of protein, 2.77% of ash, 5.35% of lipids, 3.0 mg.kg^{-1} of β-carotene, 32.2 g.100 g^{-1} of saturated fatty acids, 5.7 g.100 g^{-1} of monounsaturated fatty acids, and 55.7 g.100 g^{-1} of polyunsaturated fatty acids. The nutritional composition of *Ph. tanacetifolia* inflorescences was 91.05% of dry matter, 18.37% of protein, 15.49% of ash, 4.5% of lipids, 50.4 mg.kg^{-1} of β-carotene, 34.0 g.100 g^{-1} of saturated fatty acids, 8.8 g.100 g^{-1} of monounsaturated fatty acids, and 45.5 g.100 g^{-1} of polyunsaturated fatty acids. The prevailing amino acids investigated raw were glutamic, aspartic acid, proline, and leucine. The content of bee pollen fructose higher 44 times than inflorescences fructose. The content of maltose and lactose in both raw was less than 0.5 g.kg^{-1}. Among saturated fatty acids, the most prevailed for both bee pollen and inflorescences was palmitic acid (28.42 and 27.93 g.100 g^{-1} of fat, respectively), oleic acid (4.99 and 8.06 g.100 g^{-1} of fat, respectively) prevailed among monounsaturated fatty acids and linolenic acid (45.47 and 23.27 g.100 g^{-1} of fat, respectively) among polyunsaturated fatty acids. Bee pollen of investigated samples had the highest content of potassium (6239 mg.kg^{-1}), phosphorus (6039 mg.kg^{-1}), and sulfur (2403 mg.kg^{-1}). The obtained data can be useful in the food industry and further pharmaceutical and apicultural research.

Keywords: lacy phacelia, nutrients, fatty acids, amino acids

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Introduction

Phacelia tanacetifolia Benth. an annual herbal widely cultivated as ornamental and medicine species from Hydrophyllaceae R. Brown ex Edwards originated from North America (Pinke et al., 2022). This species is well adapted to different conditions of growth (Kizilsimsek and Ates, 2004). This is an ecological benefit plant and is well-known as a honey plant with the best quality of honey, use for mulch due to its high absorption of calcium and phosphorus in the soil, as a forage plant, prevents erosion, soil bio cleaner from nematodes (Jaramaz and Jaramaz, 2009). *Ph. tanacetifolia* is used for forage purposes as monoculture and in mixtures with for example *Pisum arvense* L. (Ates et al., 2014). The content of nutrients in different combinations of feed mixtures of *Pisum arvense* with *Ph. tanacetifolia* (75% + 25%, 50% + 50%, etc.) increased compared with the monoculture of *Ph. tanacetifolia* (Ates, 2012). Due to its numerous useful properties, *Ph. tanacetifolia* can be recommended as a multipurpose crop in the farming system (Ozkan, 2020).

Genç Lermi and Palta (2014) investigated that yield parameters and nutritive composition of raw depended on the sowing period and were higher during the autumn period. This species can be potent material for cosmetic and pharmaceutical properties due to the content of biologically active compounds (Kruk et al., 2018).

Another study demonstrated the allelopathic potential of *Ph. tanacetifolia*, however, a negative allelopathic effect was found at high concentrations, and the most effective were leaf extracts (Kliszcz et al., 2023). Also, seed meal extracts from *Ph. tanacetifolia* were not affected at 1% concentration in the soil (Restuccia and Scavo, 2023).

Bee products, namely, bee pollen has been used since ancient time and demonstrated therapeutic properties (Denisow and Denisow-Pietrzyk, 2016). Bee pollen exhibited numerous pharmacological activities such as anti-inflammatory, antioxidant, antifungal, antiviral, immunostimulant, anti-allergic, and analgesic (Saisavoey et al., 2020; Khalifa et al., 2021).

Ph. tanacetifolia is an essential source of quality nectar and pollen (Ardalani et al., 2021). Bee pollen contains flower pollen with nectar and bee secretions, is used as a health food supplement, and is a source of lipids, sugars, vitamins, proteins, amino acids, carotenoids, flavonoids, etc. (Qian et al., 2008).

Taking into account the useful properties of *Ph. tanacetifolia* raw, this study aimed to evaluate the biochemical composition of bee pollen and inflorescences of this species from the Slovak Republic that can be useful for further pharmaceutical and apicultural studies.

Material and methodology

Biological material

The plant raw material of *Phacelia tanacetifolia* Benth. collected from the experimental plots of Slovak Agricultural University in Nitra (Slovak Republic). It was collected inflorescences and bee pollen during the period of mass flowering in 2022.

Biochemical analyses

All biochemical analyses were conducted at the Slovak University of Agriculture in Nitra (Slovak Republic). All chemicals used were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and CentralChem (Slovakia).

The total dry matter, protein, ash, and lipid content determination

The total dry matter, protein, and ash were determined according to Hrytsajenko et al. (2003). Plant samples were dried in a drying oven at 105 °C till constant weight in aluminum boxes. The protein content was determined by the Kjeldahl method. The total ash content was conducted by combustion at 550 °C in the oven till constant weight. Results are given in percentages. The detailed procedures are described in Vergun et al. (2022a, 2022b). The total lipid content is conducted by the Soxhlet method with petroleum ether extraction (Hewavithrana et al., 2020). The low-boiling petroleum ether (40 °C) was used as an extractor. The difference in masses before and after the extraction process is used to calculate the total lipid content.

Analysis of sugars

Sample preparation: cornelian cherry samples of 1 g with 10 mL of water/ethanol (4 : 1) were vigorously mixed (vertical shake table; GFL, Germany). After 60 min of extraction, the mixture was centrifuged at 6000 rpm for 4 min (EBA 21, Hettich, Germany). The supernatant was filtered through the filter paper with 0.45 mm pore size (Labicom, Czech Republic) and filled up to 50 mL in a volumetric flask with water. An HPLC analysis of sugars (fructose, maltose, sucrose, lactose) was performed using an Agilent 1260 Infinity instrument (Agilent Technologies, Santa Clara, USA)

coupled to an evaporative light scattering (ELSD) detector. Separation of sugars was conducted on a Prevail Carbohydrates ES column (250 × 4.6 mm). Acetonitrile/water (75 : 25, v/v) was used as the mobile phase. The identification of sugars was made by comparing the relative retention times of sample peaks with standards (Sigma-Aldrich, Steinheim, Germany). The contents of sugars were expressed as $g.kg^{-1}$ of the dry sample.

The total carotenoid content

The total carotenoid content expressed as β-carotene was analyzed spectrophotometrically at the wavelength 440 nm (VIS spectrophotometer UV Jenway Model 6405 UV/VIS). Sample (1 g) was disrupted with sea sand and extracted with acetone until complete discoloration. Petroleum-ether was added and then water, in purpose to the separation of phases. After the separation, the petroleum ether-carotenoid phase was obtained, and the absorbance was measured (ČSN 560053, 1986).

Analysis of amino acid profile

Amino acids were determined according to the standard procedure of AOAC (1990). The bee pollen samples were analyzed in the following way: were undergone a hydrolysis process in 6 N hydrochloric acid (HCl) at 110 °C for 24 h followed by the reconstitution of the samples with physiological buffer (0.12 N, pH 2.2) and analyzed by the amino acid analyzer (Model AAA-400, Ingos, Czech Republic)) associated with LCA K07/Li (PEEK column 4.6 × 150 mm) column.

Analysis of fatty acid composition

Lipid fraction extracted from each morphological part of *Cornus mas* was determined as follows: the samples were prepared according to official methods Ce 2-66 (1997) to convert triacylglycerols into methyl esters of fatty acid (FAMEs). The FAMEs were analyzed by gas chromatography using an Agilent 6890N instrument (Agilent Technologies, Santa Clara, USA) equipped with a flame ionization detector (FID; 250 °C; constant flow, hydrogen 40 $mL.min^{-1}$, air 450 $mL.min^{-1}$), a capillary column DB-23 (60 m × 0.25 mm, film thickness 0.25 µm, Agilent Technologies, Santa Clara, CA, USA). A detailed description of the chromatography conditions is presented in the work of Szabóová et al. (2020). Standards of a C4-C24 FAME mixture (Supelco, Bellefonte, PA, USA) were applied to identify FAME peaks. The evaluation was carried out by the ChemStation 10.1 software. The contents of FAs were expressed as $g.100\ g^{-1}$ of lipids.

Elemental analysis

The contents of macroelements, microelements and trace metals were determined by the inductively coupled plasma optical emission spectroscopy (ICP-OES) according to Divis et al. (2015) by using an ICP-OES instrument (Ultima 2, Horiba Scientific, France). Samples were prepared for analysis after microwave digestion (Milestone 1200, Milestone, Italy), 0.25 g of sample was decomposed in a mixture of nitric acid (6 mL) (Analytika Praha Ltd, Czech Republic) and hydrochloric acid (2 mL) (Analytika Praha Ltd, Czech Republic). After the decomposition sample was filtered through filter paper (0.45 mm pore size) and filled up to 25 mL in a volumetric flask with pure water.

Statistical analysis

The results are expressed as mean values of three replications ± standard deviation (SD). Data were analyzed with the ANOVA test and differences between means were compared through the Tukey-Kramer test ($p < 0.05$).

Results and discussion

The biochemical composition is one of the most important parameters in the evaluation of plant raw material that depends on the stage of growth, conditions of development, species, genotypes, etc. (Vergun et al., 2022a). The content of dry matter, protein, ash, lipids, β-carotene, ascorbic acid, fatty acid, and others is a basic nutritive composition evaluation of plant raw material (Sharma and Kaushik, 2021; Vergun et al., 2022b). Plants produce the phytochemicals that protect them from diseases. The soul food source for many insects is floral nectar and pollen (Chlebo and Adamchuk, 2017; Palmer-Young and Thursfield, 2017). The nutritional composition of bee pollen is carbohydrates, proteins, lipids, vitamins, minerals, and polyphenols, that exhibit numerous biological activities (Arruda et al., 2013; Li et al., 2018; Ghosh and Jung, 2020). The biochemical composition of bee pollen depends on plant origin, ecological conditions, and conditions after collecting (Denisow and Denisow-Pietrzyk, 2016). Thakur and Nanda (2020) summarized data from numerous studies of the biochemical composition of bee pollen and reviewed the content of carbohydrates from 18.50 to 84.25%, proteins from 4.50 to 40.70%, lipids from 0.41 to 13.50%, fibre from 0.15 to 31.26%, ash from 0.50 to 7.75%, phenolic compounds from 0.69 to 213.0 mg $GAE.g^{-1}$, etc., depended on plant species.

In this study, *Ph. tanacetifolia* bee pollen had 73.3% of dry matter, 27.44% of protein, 2.77% of ash, 5.35%

of lipids, 3.0 mg.kg^{-1} of β-carotene, 32.2 g.100 g^{-1} of saturated fatty acids, 5.7 g.100 g^{-1} of monounsaturated fatty acids, and 55.7 g.100 g^{-1} of polyunsaturated fatty acids (Figure 1). The nutritive composition of *Ph. tanacetifolia* inflorescences was 91.05% of dry matter, 18.37% of protein, 15.49% of ash, 4.5% of lipids, 50.4 mg.kg^{-1} of β-carotene, 34.0 g.100 g^{-1} of saturated fatty acids, 8.8 g.100 g^{-1} of monounsaturated fatty acids, and 45.5 g.100 g^{-1} of polyunsaturated fatty acids.

As reported Singh et al. (1999), the lipid content of *Brassica campestris* L., *Cosmos bipinnatus* Cav., and *Raphanus sativum* L. pollen was 20.3, 19.4, and 17.8%, respectively. Human and Nicolson (2006), found that the bee pollen composition of *Aloe greatheadii* var. *davyana* depended on condition after collection. In this case, fresh pollen, bee-collected pollen, and stored pollen had different protein, lipid, ash, and carbohydrate content. The most content of protein, ash, and lipids had samples of fresh pollen and carbohydrates the samples of storage pollen. According to Addi and Lamessa (2009), the study of the nutritive composition of numerous bee pollen of different plant species from twelve families showed the accumulation of 7.87–28.68% of crude protein. The experiment with the use of *Ph. tanacetifolia* as feed culture showed that cows produced milk with 598 g.kg^{-1} of saturated fatty acids, 350 g.kg^{-1} of monounsaturated fatty acids, and 52.1 g.kg^{-1} of polyunsaturated fatty acids, whereas feed phacelia contained saturated, monounsaturated, and polyunsaturated fatty acids 263. 97, and 641 g.kg^{-1}, respectively (Käbler et al., 2011). The study of different species showed that fatty acid content varied from 0.52 to 8.21%. Fatty acids account for 3% of the total lipid content of pollen grains (Gercek et al., 2022). As reported Denisow and Denisow-Pietrzyk (2016), the β-carotene content of bee pollen can be from 0.01 to 0.20 g.kg^{-1} (10–200 mg.kg^{-1}). Bee pollen of *Taraxacum officinale* L. had a lipid content 19.04% (Prđun et al., 2021).The study of selected plant species showed that geographical origin affected the fatty acid composition of bee pollen (Liolios et al., 2022).

Amino acids are one of the important biochemical components of bee pollen. According to Bayram et al. (2021), studied bee pollen samples showed the predominant content of proline, asparagine, and aspartic acid. A high concentration of proline, in this case, can be the parameter of sample freshness. The quantitive and qualitative amino acid content of bee pollen depends on numerous factors among which is harvesting season (Al-Kahtani et al., 2020).

As shown in Figure 2, the amino acid content of bee pollen from *Ph. tanacetifolia* decreased in the following order: glutamic acid > aspartic acid > proline > lysine > leucine > glycine > alanine > arginine> phenylalanine. The rest amino acids had a concentration of less than 10 g.kg^{-1}.

Silva et al. (2014) determined the amino acid composition of *Senna* spp. and detected serine and proline as prevailed in pollen. According to Ghosh et al. (2020), bee pollen of selected plant species accumulated the most glutamic acid (2.65 g.100 g^{-1} for *Trifolium repens* L. and 1.29 g.100 g^{-1} for *Coreopsis drummondii* (D.Don) Torr & A. Gray) and lysine (1.17 g.100 g^{-1} for *Erigeron annus* (L.) Pers. and 1.12 g.100 g^{-1} for *Oenothera biennis* L.).

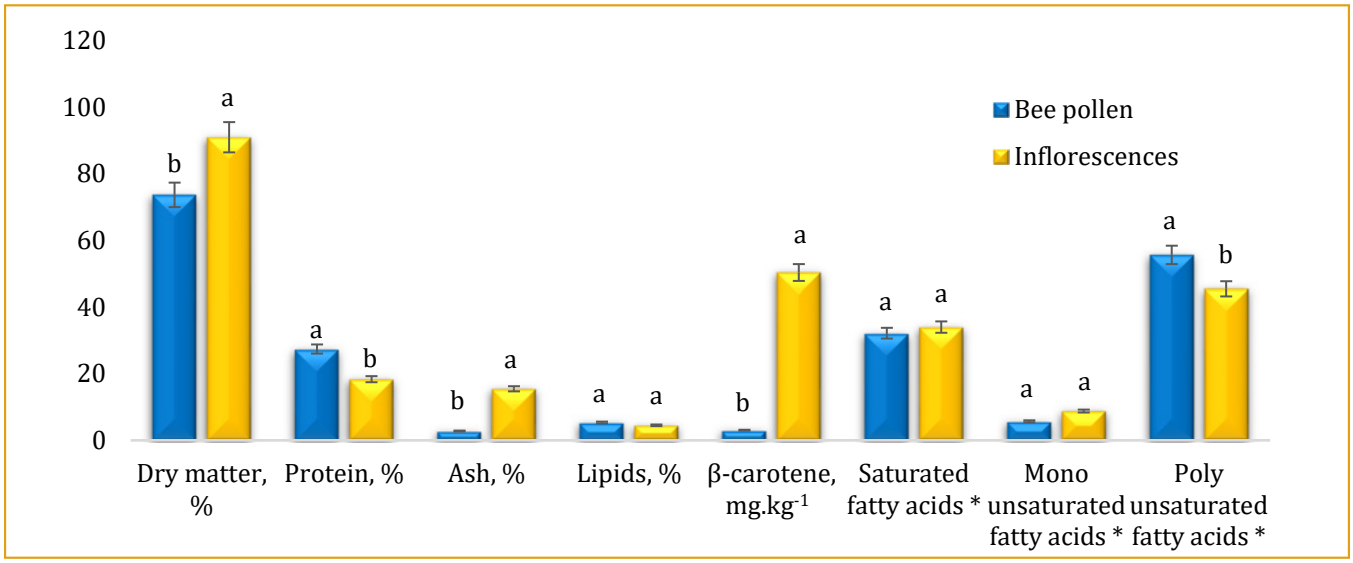

Figure 1 The nutritive composition of bee pollen and inflorescences of *Phacelia tanacetifolia* Benth.
* – g.100 g^{-1} of fat. Means in each column followed by different letters are significantly different (p <0.05)

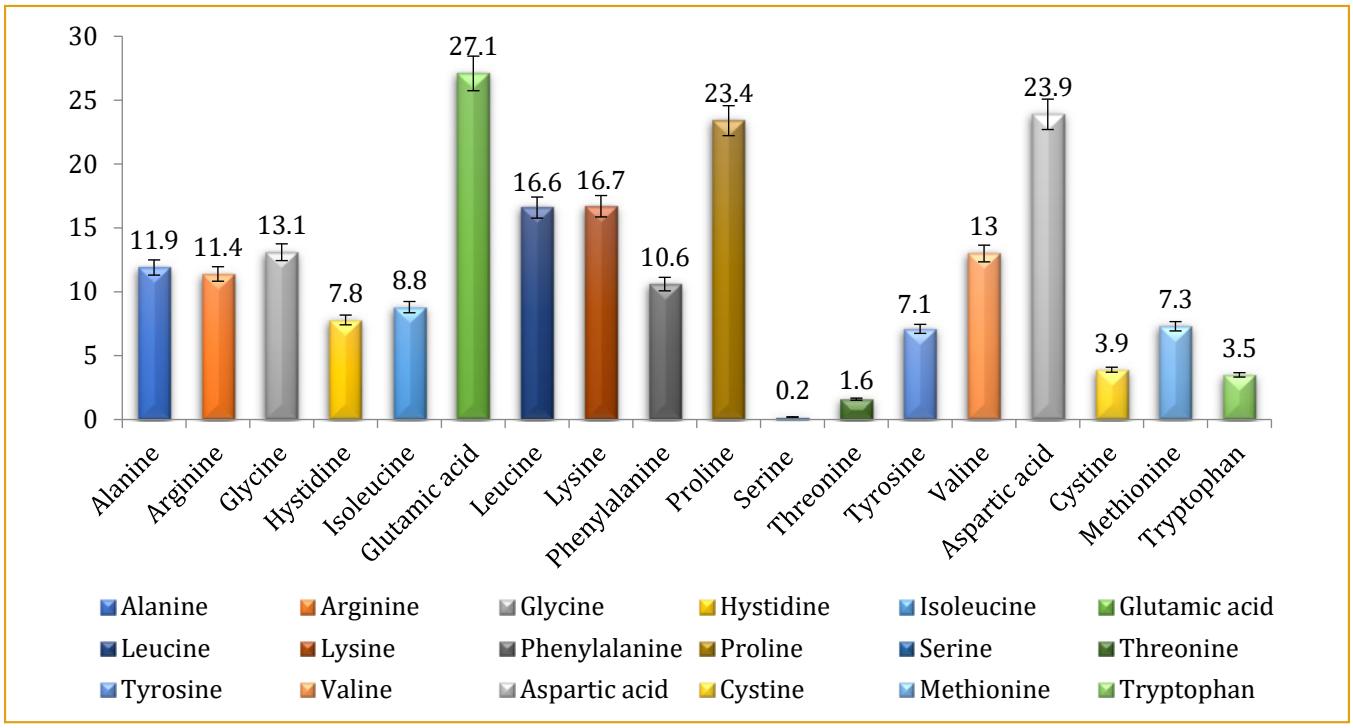

Figure 2 The protein composition of bee pollen of *Phacelia tanacetifolia* Benth., g.kg^{-1}

Figure 3 demonstrated that the content of amino acids in flower extracts decreased in followed order: glutamic acid > aspartic acid > leucine > phenylalanine > lysine > valine > glycine, proline > isoleucine. The rest of the amino acids accumulated in quantity less than 7.0 g.kg^{-1}.

The content of proline in the three honey compositions with *Ph. tanacetifolia*, as reported by Horčinová Sedlačková et al. (2022), was 224.38–296.21 mg.kg^{-1}.

One of the most important functions of plant sugars is the regulatory role during growth and development and it depends on numerous factors such as cold or drought stress, pathogens, phosphorus deficiency, and peculiarities of growth namely increased sugar demand in different plant tissues (Ciereszko, 2018). The plant sugars also with gene expression translate the nutrient status at the different periods of growth (Stephen et al., 2021). Sugars are distributed in the different

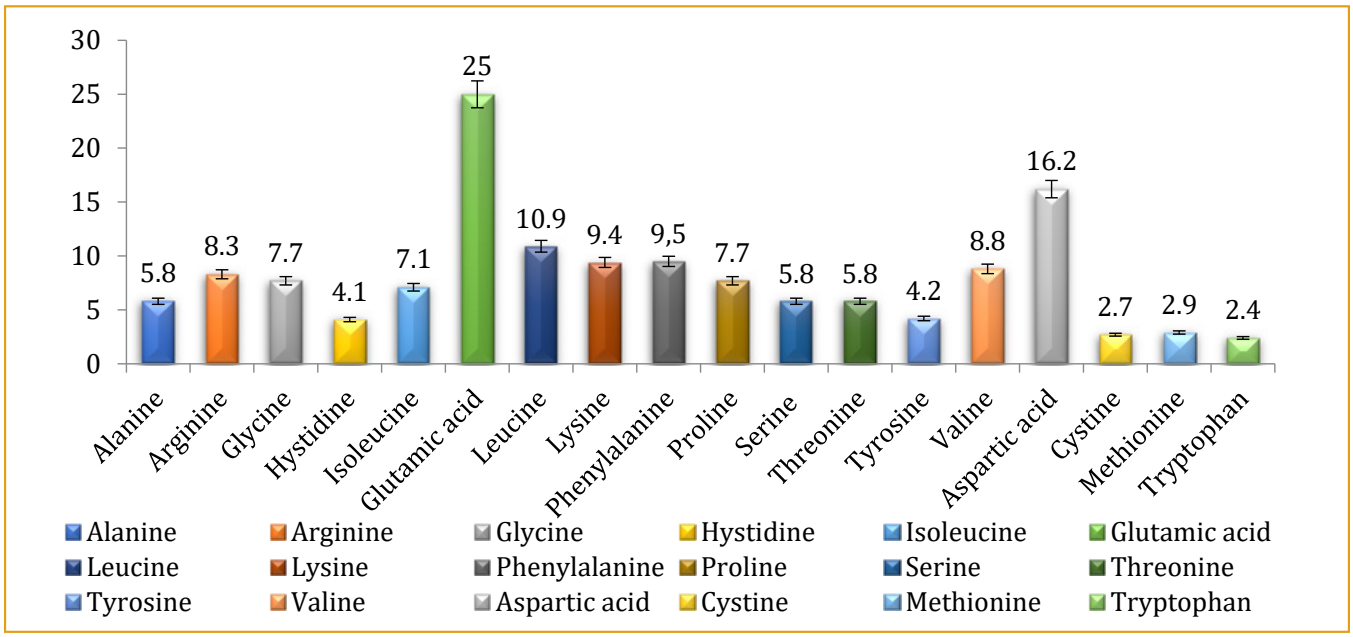

Figure 3 The protein composition of inflorescences of *Phacelia tanacetifolia* Benth., g.kg^{-1}

Table 1 The content of saccharides in bee pollen and inflorescences of *Phacelia tanacetifolia* Benth. (g.kg^{-1} of dry weight; mean ±SE)

Saccharide	Bee pollen	Inflorescences
Fructose	174.7 ±3.12	3.9 ±0.21
Maltose	<0.5	<0.5
Saccharose	<0.5	4.2 ±0.11
Lactose	<0.5	<0.5

plant parts, including inflorescences and flowers that produce nectar which mainly includes fructose, glucose, sucrose, etc. (Adamchuk et al., 2017; Gardana et al., 2018).

The content of saccharides of *Ph. tanacetifolia* bee pollen is represented in Table 1. The content of bee pollen fructose higher 44 times than inflorescences fructose. The content of maltose and lactose in both raw was less than 0.5 g.kg^{-1}.

Thakur and Nanda (2020) reviewed that different bee pollen glucose content was from 2.77 to 28.49 g.100 g^{-1}, fructose from 4.9 to 33.48 g.100 g^{-1}, sucrose from 0.05 to 9.02 g.100 g^{-1}, etc, depended on plant species. According to Horčinová Sedlačková et al. (2022), the sucrose content of honey composition with *Ph. tanacetifolia* was 0.84–7.37%. In this case, the highest sucrose content was found in composition with *Aesculus hippocastanum* L. and *Robinia pseudoacacia* L.

The fatty acid composition of pollen is one of the most important biochemical parameters. Among saturated fatty acids, the most prevailed for both bee pollen and inflorescences was palmitic acid (Table 2). Oleic acid prevailed among monounsaturated fatty acids and linolenic acid among polyunsaturated fatty acids.

It should be noted that fatty acids such as C8:0, C20:4, C22:1, and C24:1 are determined as nondominant and were in quantity less than 0.1 mg.kg^{-1}. Hsu et al. (2021) studied 11 different bee pollen samples and resulted also the prevailed palmitic and linoleic acids. Al-Kahtani et al. (2021) determined that the lipid and fatty acid content of *Brassica napus* L., *Medicago sativa* L., *Helianthus annuus* L., etc., depended on

Table 2 The fatty acid composition of bee pollen and inflorescences of *Phacelia tanacetifolia* Benth. (mg.kg^{-1} of dry weight; mean ±SE)

Fatty acid	Bee pollen	Inflorescences
Saturated fatty acid		
Capric acid (C10:0)	0.34 ±0.02	<0,1
Lauric acid (C12:0)	0.23 ±0.01	0.19 ±0.01
Myristic acid (C14:0)	1.37 ±0.01	1.86 ±0.12
Palmitic acid (C16:0)	28.42 ±1.13	27.93 ±1.24
Heptadecanoic acid (C17:0)	<0.1	0.21 ±0.01
Stearic acid (C18:0)	0.93 ±0.05	2.79 ±0.21
Arachidic acid (C20:0)	0.25 ±0.02	0.64 ±0.05
Behenic acid (C22:0)	0.23 ±0.01	0.37 ±0.01
Monounsaturated fatty acid		
Palmitoleic acid (C16:1)	0.66 ±0.02	0.35 ±0.01
Heptadecenoic acid (C17:1)	<0.1	0.77 ±0.04
Oleic acid (C18:1)	4.99 ±0.62	8.06 ±0.35
Eicosenoic acid (C20:1)	<0.1	0.36 ±0.02
Polyunsaturated fatty acid		
Linoleic acid (C18:2)	10.19 ±0.54	22.26 ±0.78
Docosadienoic acid (C22:2)	1.47 ±0.21	<0.1
Linolenic acid (C18:3)	45.47 ±1.23	23.27 ±0.92

Table 3 Element composition of bee pollen and inflorescences of *Phacelia tanacetifolia* Benth. (mg.kg^{-1} of dry weight; mean ± SE)

Element	Bee pollen	Inflorescences
	Macroelements	
K	6239 ±350	27038 ±546
P	6039 ±267	6624 ±234
Ca	1067±76	33282 ±215
S	2403 ±123	4068 ±156
Mg	553±32	4513 ±173
Na	18.02 ±0.21	601 ±31.12
	Microelements	
Zn	55.01 ±2.12	45.13 ±2.51
Fe	56.21 ±1.54	45.25 ±1.76
Cu	9.03 ±0.23	12.02 ±0.65
Mn	21.9 ±0.56	36.8 ±0.43
Cr	<0.2	0.44 ±0.02
	Metals	
Al	2.42 ±0.11	8.05±0.22
As	<0.3	<0.3
Cd	0.045 ±0.001	0.210 ±0.06
Hg	0.003 ±0.0001	0.008 ±0.0001
Pb	0.10 ±0.001	0.75 ±0.002

seasonal collection. The palmitic, stearic, oleic, and linolenic acids have prevailed in this case.

Bioelements including macro- and micronutrients present in the bee pollen in quantity of 1.6% (Komosinska-Vassev et al., 2015). The composition of macro-, microelements, and metals in the bee pollen and inflorescences of *Phacelia tanacetifolia* Benth. were significantly different mostly depending on the sample (Table 3). Bee pollen of investigated samples had the highest content of potassium, phosphorus, and sulfur. Investigated inflorescences had a higher content of most elements than bee pollen, especially K, Ca, Na, and Mg.

Matuszewska et al. (2021) determined that investigated bee pollen and royal jelly contained a high content of potassium, phosphorus, and sulfur which is similar to our study. According to Valverde et al. (2023), the study of seventy-one samples showed that terms of the apiary and harvesting had no affected on the elemental composition of bee pollen. Phosphorus and potassium have prevailed elements in this case, the same as in the present study. Potassium and phosphorus are two of the most essential elements of bee pollen which are contained in the most quantity along with other elements (Ghouizi et al., 2023).

Conclusions

Taking into account obtained data, it should be noted that bee pollen and inflorescences of *Ph. tanacetifolia* from the Slovak Republic are rich sources of nutrients and have a useful biochemical composition. The raw bee pollen and inflorescences had a high content of dry matter, ash, lipids, β-carotene, saturated, unsaturated fatty acids, and macro- and microelements. Additionally, the proteins of bee pollen of *Ph. tanacetifolia* and its inflorescences had high concentrations of glutamic, aspartic acid, proline, and leucine. The content of bee pollen fructose higher 44 times than inflorescences fructose. Among saturated fatty acids, the most prevailed for both bee pollen and inflorescences was palmitic acid. Oleic acid prevailed among monounsaturated fatty acids and linolenic acid among polyunsaturated fatty acids. Bee pollen of investigated samples had the highest content of potassium, phosphorus, and sulfur, while inflorescences had a higher content of potassium, calcium, sodium, and magnesium. The obtained data concerning the biochemical composition of bee pollen and inflorescences of *Ph. tanacetifolia* can be useful in the pharmaceutical, food, apicultural, and cosmetic industries.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical statements

This article does not contain any studies that would require an ethical statement.

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