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Bee collected pollen as a value-added product rich in bioactive compounds and unsaturated fatty acids: A comparative study from Turkey and Romania

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ABSTRACT

The aim of this study was to determine the botanical origin, phenolics, flavonoids and fatty acids content, antioxidant and antimicrobial properties, of 18 bee collected pollen (BP) samples from Turkey and Romania. Five plant families with predominant pollens (>45%) were found in the Turkish samples, and only three in the Romanian samples. The phenolic content, varied between 16.40 and 41.17 mg GAE/g and the flavonoids content varied between 2.39 and 7.17 mg QE/g. The highest value of DPPH was 2.93 mmol Trolox/g and 9.64 mmol Trolox/g for the TEAC, whereas the IC_{50} value of α-Amylase inhibition was 8.10 mg/mL. We also verified that the presence of the methanolic extract of BP differentially affected the growth of Gram-positive and Gram-negative bacteria under study, strongly depending on the microorganism and the botanical origin of the BP samples used. The fatty acids contents were closely correlated with the above-mentioned parameters especially with the botanical origin and antibacterial activity. Our findings suggest that BP is a rich source of unsaturated fatty acids and bioactive compounds, which can be considered a value-added product. Furthermore, the differences in Turkish and Romanian BP chemical composition is also shown based on their antimicrobial and α-amylase inhibitory activities.

1. Introduction

Globally, increasing honey consumption to treat severe coughs and cold-induced by COVID-19 and the seasonal flu is anticipated to boost market growth. In addition, the demand for immunity-boosting products, such as bee collected pollen (BP) and beebread has increased since the pandemic started. Regarding the global bee products production, Turkey is the 2nd largest global producer of honey after China and to a lesser extent for pollen, whereas Romania ranks 15th in the global production of honey and lowest in BP. Due to the COVID-19 pandemic, Turkey reported an increasing demand for bee products in the last years. The number of hives reached 8 million as of this year, and honey production increased from 81 to 110 thousand t. This year, the Mersin Beekeepers Association President Adam Kurt stated that: "Our bee products have experienced an increase in demand by 350%. They are products that strengthen the immune system and people feel safer against COVID-19 by consuming them. The first honey harvest in our province was sandalwood from Erdemli district of Mersin, which is good for stomach pain and/or disorders and intestinal diseases" (www.tridge.com). Furthermore, an increase in BP demand was noticed, as well as in the production (>200 t/year). According to the statistics from 2016, Romania was the leading country in honey production. Last year, in Romania 24 thousand t of dry pollen and 48 thousand t of fresh pollen were harvested and the number of hives reached 2 million (http://www.icdapicultura.ro/apicultura-in-uniunea-europeana-2016/). The pollen grains are moistened with salivary secretions and nectar to form a pellet

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and after carried as pellets using the pollen basket and finally stored inside the hive for further consumption to accomplish the requirements for protein (Thakur & Nanda, 2020). Thanks to its high nutritional content and chemical structure, BP is also used as a food supplement in human nutrition (Bobiş et al., 2010). For this purpose, using the pollen traps attached to the hive entrance, the pollen loads on the leg of the bees are collected and presented to the consumer.

Examination of plant's pollen under the microscope is carried out by palynological methods. The fact that each plant species and even some subspecies have their own pollen morphology allows their identification under the microscope (d'Albore, 1997). In this way, it is also possible to examine plant pollens in bee products by palynological methods and to determine the botanical origin of the product.

BP is promoted as a health food with a wide range of nutritional and therapeutic properties (Eraslan et al., 2009), while more recent studies confirm their use as food supplements due to their bioproperties, which support their future use as medicines (Campos, Frigerio, Lopes, & Bogdanov, 2010; Margaoan et al., 2019). Furthermore, due to its high concentration in reduced sugars, unsaturated and saturated fatty acids, as well as minerals like Cu, Fe, Zn and a high Na/K ratio makes BP a value-added product important for human diets (Almeida-Muradian, Pamplona, Coimbra, & Barth, 2005; Campos et al., 2008; Thakur and Nanda, 2020; Mărgăoan et al., 2012). The chemical composition of BP strongly depends on the plant source (being a mixture of BP floral pollens) and geographical origin, together with other factors such as climatic conditions, soil type and processing methodologies (Campos et al., 2008; Szczesna, 2002). Regarding its composition, there are about 250 substances including amino acids, lipids (highlighting its nutraceutical importance as a source of ω-6 and ω-3 polyunsaturated fatty acids), phenolic compounds, vitamins, macro- and micronutrients (Komosinska-Vassev, Olczyk, Kaźmierczak, Mencner, & Olczyk, 2015; Conte et al., 2017; Margaoan et al., 2019).

BP with its high nutritional value and bioactive compounds, has a positive effect on human health, and therefore, is regarded as a "functional food" (Kostić et al., 2020). These features strengthen the body's immunity, help it to fight bacteria, ensure the body energy, as well as quality tissue repair (Bobiş et al., 2010). Furthermore, pollen loads color is determined by the presence of pigments (i.e. flavonoids and/or carotenoids), along with antioxidants and other elements, compounds that extensively contribute to the potential bioactivities of BP (Margaoan et al., 2019). It has been shown that the type and concentration of polyphenolic compounds found in BP strongly influence the antibacterial and antioxidant activity (Morais, Moreira, Feás, & Estevinho, 2011). As healthful characteristics, multiple studies have demonstrated that BP has anti-anemic, restorative hormone and intestinal regulator, vascular protector, hepatoprotective, anti-atheroscleorotic agent, antiallergic, anticarcinogenic, antioxidant, antibacterial and antifungal properties (Campos, Frigerio, Lopes, & Bogdanov, 2010; Velásquez et al., 2017).

The phenolic compounds are beneficial for human health since they decrease the risk of degenerative diseases by reducing oxidative stress (Morais et al., 2011). Lipids are important to honeybees, firstly as a source of energy with some components of lipids involved in the synthesis of reserve fat, glycogen and cells membrane structure. The selection of essential fatty acids (EFAs) and antioxidants found in BP is significant for growth, development and disease prevention in humans. Several biological functions require EFAs for regulated levels of plasma lipids, insulin activity, cardiovascular and immune function to ensure better health (Thakur & Nanda, 2020). Fatty acids (FAs) and sterols are also significant in the nutrition, development and reproduction of honeybees. Lipids vary greatly in concentration in different plant species pollen (Margaoan et al., 2014; Szczęsna, Rybak-Chmielewska, & Chmielewski 2002; Conte et al., 2017; Bastos et al., 2004). The literature shows a high variation in BP lipid content according to their botanical and geographical origins.(T'ai and Cane, 2000), showed that ether-extractable material from dry pollen of 62 plant species ranges between 0.8% and 18.9%. The total lipid fraction of BP (monofloral and multifloral) contains high levels of long-chain FAs, the most abundant being linoleic [(18:2 ω-6)], α-linolenic [(18:3 (ω-3)] and palmitic (16:0) acids (Szczęsna, 2006).

Combining BP with high levels of α-linolenic acid, as well as near 1:1 ratio of ω-6 to ω-3 polyunsaturated fatty acids (PUFAs) confirms its use as a balanced source for human health. These acids exert a variety of health benefits, BP having a higher ω-3 acid value than most vegetables (Margaoan et al., 2019).

The antibacterial activity of BP is due to flavonoids, polyphenols and FAs content (Didaras, Karatasou, Dimitriou,). For the antibacterial activity the quality of flavonoids and polyphenols proves to be significant, detrimental to the quantity. Although there is high variability of BP antimicrobial's activity, it demonstrates higher antimicrobial activity against Gram-positive compared to Gram-negative bacteria (Morais et al., 2011; Didaras et al., 2020). Several FAs such as capric, lauric, myristic, linoleic and linolenic are known to have significant antimicrobial properties, some being inhibitorier than others, whereas FAs such as palmitic, stearic, and oleic proved to be antimicrobial inactive (Manning, 2001).

As with all bee products, the nutritional and chemical content of BP is also affected by many parameters such as botanical origin, climate, geographical factors and storage conditions (Morais et al., 2011; Mărgăoan et al., 2014; Mayda, Özkök, Bayram, Gerçek, & Sorkun, 2020). For this reason, determining the botanical origin is significant when evaluating the chemical properties of BP. In the present study, our main objectives were to demonstrate if there is a correlation between the FAs content and botanical origin, as well as between the total polyphenols, flavonoids and antibacterial activities.

We investigated the botanical origin, antioxidant activity, α-amylase inhibition, total polyphenols and flavonoids, FAs content and antibacterial activities of BP samples collected from Turkey and Romania. Furthermore, the relationship between FAs and the aforementioned analysis was evaluated by means of principal component analysis, hierarchical clustering and heat-map.

2. Materials and methods

2.1. Bee collected pollen samples

We assessed 18 samples from the Marmara and Black Sea Coast regions, Turkey and from the north-west of the Transylvania area, Romania. Each sample was taken directly from the apiaries of the beekeeper (Supplementary Table S1).

2.2. Determination of botanical origins of bee collected pollen samples

The methods described by Louveaux Maurizio, & Vorwohl, 1978 and Almeida-Muradian et al., 2005, were used for the preparation of pollen slides. From each sample (2 g), one microscopic slide was prepared without acetolysis by dissolving and washing the pollen in diluted H_2SO_4 (5‰). Slide examination was performed using an Olympus BX51 optical microscope at 400 × magnification. Pollen frequencies (%) of the BP samples were determined according to the methodology described by Louveaux Maurizio, & Vorwohl, 1978 and adapted for BP The different pollen types identified were divided into four classes: predominant pollen (>45% of the total pollen detected in honey); secondary pollen (16–45%); minor important pollen (3–15%); minor pollen (<3%) (Louveaux, Maurizio, & Vorwohl, 1978).

2.3. Preparation of extracts

The BP samples (2.5 g) were extracted separately for three times with 5 mL methanol solvent (80:20 *v/v*, methanol:water) for 1 h at room temperature. After sonication (Bandelin RK 100 H) for 15 min at 320 W, temperature of 36 °C and maceration the aliquot was centrifuged (15269×*g*), for 10 min and evaporated to dryness under vacuum. The

resulting extracts were dissolved in methanol and stored until analysis (4 °C).

2.4. Determination of total phenolic content(TPC) and total flavonoid content (TFC)

TPC were determined by the Folin–Ciocalteau method (Attard, 2013). Briefly, diluted 1:10, 100 μL Folin–Ciocalteu reagent with deionised water (0.2 M—with respect to acid), was added to 10 μL of BP extracts and mixed with 80 μL sodium carbonate (Na_2CO_3) solution (1 M). The absorbance of the resulting mixture was measured at 630 nm, after 20 min in darkness. The assays were run in triplicate and expressed as mg of GAE (gallic acid equivalents) per g of extract.

TFC were measured by the aluminum chloride colorimetric assay developed by (Marghitas et al., 2009) and adapted for the 96 well microplate reader (Synergy™ HT BioTek Instruments, USA), using quercetin as reference standard. A volume of 25 μL diluted sample was added to 100 μL distilled water and 10 μL sodium nitrate ($NaNO_2$) solution 5g/100 mL. After 5 min to the mixture, 15 μL aluminum chloride ($AlCl_3$) 10% and 50 μL sodium hydroxide 1 M (NaOH) was added. Thereafter, 50 μL of distilled water was added and rigorously mixed. The results were expressed as mg of QE (quercetin equivalent) per g of extract.

2.5. Determination of DPPH scavenging activity

The scavenging activity (H/e-transferring ability) of BP extracts against 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) was evaluated by a modified method of Velázquez, Tournier, De Buschiazzo, Saavedra, and Schinella (2003) adapted for the 96 well microplate reader. Briefly, an aliquot (40 μL) of diluted BP extracts was mixed with 200 μL $DPPH^{\bullet}$ solution (0.02 mg/mL). The samples were kept at room temperature for 15 min and after the absorbance were measured at 517 nm. Absorbance of blank sample containing the same amount of solvent (methanol: water, 80:20 *v/v*) and DPPH radical scavenge activity was prepared and measured at 517 nm. The percentage of absorbance inhibition was calculated using the equation:

$$Scavange \cdot (\%) \cdot = \cdot \left(1 - \frac{Asample}{Ablanck}\right) \cdot \times \cdot 100$$

The radical scavenging activity was expressed in millimol of Trolox equivalents per gram of sample (mmol Trolox/g dry matter sample).

2.6. Determination of Trolox equivalent antioxidant capacity (TEAC)

For Trolox equivalent antioxidant capacity assay, the procedure followed the method of Re et al. (1999). The TEAC assay is based on the scavenging of the 2,2′-azinobis-(3- ethylbenzothiazoline- 6-sulphonic acid) radical cation (ABTS•+) converting it into a colourless product. The $ABTS^{\bullet+}$ cation radical was produced by the reaction between 7 mmol/L ABTS solution and 2.45 mmol/L potassium persulfate solution. Before usage, the $ABTS^{\bullet+}$ solution was diluted to get an absorbance of 0.700 ± 0.025 at 734 nm with ethanol. For the assay the resulting solution was mixed with 17 μL of sample. For the control, the sample volume was replaced with an equivalent volume of solvent (methanol: water, 80:20, *v/v*), the rest of the method being the same. The extent of inhibition of the sample was calculated using the formula mentioned above, compared with a standard curve of Trolox (0.4–0.04 mmol/L). Results were expressed in millimol of equivalent Trolox per gram of sample (mmol Trolox/g dry matter sample).

2.7. Determination of α-amylase inhibition

α-Amylase activity was assayed in the presence of soluble starch as substrate. The DNS method described by Bernfeld (1955) was used for reducing ends as glucose equivalent. Reaction mixture containing 300 μL of 1g/100 mL soluble starch and 300 μL of enzyme solution was incubated at 35 °C for 30 min. DNS reagent was added into tubes at an equal volume and kept in a water bath. Absorbance was measured at 550 nm, by using acarbose as a reference inhibitor (Keskin, Şirin, Çakir, & Keskin, 2019).

2.8. Antimicrobial activity

The antibacterial activity of the BP samples was tested *in vitro* against the Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538P and the Gram-negative bacterium *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922. Briefly, the minimum inhibitory concentrations (MIC values) were determined using the 96 well microplate reader (Synergy™ HT BioTek Instruments, USA). Bacteria were grown in liquid nutrient broth (Merck Darmstadt, Germany, 105443) at 37 °C with constant agitation, to prepare standardized inoculums. The optical density has been adjusted to 0.5 on McFarland standard. BP extracts were diluted in dimethylsulfoxide (DMSO) and the concentrations ranged between 1 and 50 mg/mL. From each extract dilution a volume of 100 μL was added to the same volume of broth medium and inoculated with 10 μL of the selected bacterial species. Under continuous shaking, the plates were incubated for 24 h at 37 °C in a spectrophotometer multichannel (BioTek Instruments, Winooski, VT, USA) and measured every 15 min at an optical density of OD600 nm. In each experiment a positive (Gentamicin) and a negative control (DMSO) were used. The final volume of all incubated samples was 200 μL. The results were expressed as mg/mL.

2.9. Fatty acids analysis

The total lipids (TLs) of the bee-collected pollen samples were extracted using a chloroform/methanol mixture (2:1, *v/v*) (Folch, Lees, & Stanley, 1957). Following all extraction steps and drying procedure, the recovered TLs fractions were and stored at −18 °C for further analysis.

The fatty acid methyl esters (FAMEs) were prepared from the TLs using the acid-catalyzed transesterification procedure described by (Dulf, 2012). The FAMEs were determined by gas chromatography−mass spectrometry (GC-MS). A PerkinElmer Clarus 600 T GC-MS equipped with a Supelcowax 10 (60 m × 0.25 mm i. d., 0.25 μm film thickness) capillary column was used. Helium was used as the carrier gas. The injector temperature was 210 °C and for the column was: 140 °C initial temperature increased by 5 °C/min to 220 °C and hold for 20 min. The injected volume was 0.5 μL. The positive ion electron impact (EI) mass spectra were recorded at an ionization energy of 70 eV and a trap current of 100 μA with a source temperature of 150 °C. Identification of FAMEs was accomplished by comparing their retention times with known standards (Supelco no. 47885-U, 37 component FAME Mix). The amount of fatty acids was expressed as percent of total fatty acids.

2.10. Statistical analysis

All determinations were made in series of three independent repetitions and the obtained results were expressed as average ± standard deviation (SD). Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Kruskal-Wallis test using XLSTAT software (Addinsoft, New York, NY). Moreover, we performed principal component analysis (PCA) using the Paleontological Statistics (PAST) software (Hammer, Harper, & Ryan, 2001). Heatmap and dendrograms were generated using the Euclidean distance based on Ward's algorithm for clustering (Ward, 1963).

3. Results and discussion

3.1. Botanical origin of bee collected pollen samples

The pollen spectrum analysis of the samples from Romania and Turkey with the identified plant families and species giving the predominant, secondary, and minor pollen in the analyzed samples are exemplified in Supplementary Table S1. According to the palynological analysis, the majority of the samples were found to be multifloral, with the exception of BP1 TR, 3 TR and 7 TR which proved to be monofloral with a dominance of a specific family exceeding 80%, as well as BP7 RO predominant in Rosaceae. The pollen type diversity ranged between 3 and 19 per sample. The highest diversity was observed in sample BP9 TR (multifloral) and the lowest diversity in BP3 TR (Asteraceae). The samples from Turkey were predominant (>45%) in Asteraceae, Brassicaceae, Fabaceae (*Coronilla* sp.), Fagaceae (*Castanea* sp.) and Lamiaceae. As secondary pollens, the families Asteraceae, Brassicaceae, Fagaceae (*Castanea* sp.), Fabaceae (*Coronilla* sp. and *Onobrychis* sp.) and Rosaceae (*Pyrus* sp.) were found. From the Romanian samples we identified 16 pollen types, from which Fabaceae, Fagaceae (*Castanea* sp.) and Rosaceae (*Filipendula* sp.) were identified in more than 50% of the samples. As secondary pollens, the families Brassicaceae (*Brassica* sp.), Cornaceae (*Cornus* sp.), Fabaceae, Fagaceae (*Castanea* sp.) and Salicaceae (*Salix* sp.) were found in the samples from Romania (16–45%). Further details can be seen in Supplementary Table 2. Pollen botanical origin from the pollen pellets may vary according to the region of collection, vegetation available for bees at the collecting moment. Furthermore, a cluster analysis was performed in order to differentiate the BP samples based on their botanical and geographical origin ($r = 0.91$). As shown in Fig. 1, the dendrogram obtained from the Cluster analysis (Euclidean distance) reveals the formation of three groups. The 1st cluster is composed of samples BP8 RO, BP7 TR and BP9 TR predominant in Fagaceae. The second group mainly corresponds to the Romanian samples except samples BP1 TR, BP8 TR and BP10 TR. This is explained by the sample's predominance in Fabaceae and Rosaceae families. The third group comprises the samples BP4 RO along with samples BP2-6 TR predominant in Asteraceae and Brassicaceae. This approach shows that cluster analysis can discriminate the BP samples based on their geographic botanical analysis.

3.2. Total phenolic and flavonoid content of bee pollen samples

Polyphenolic and flavonoid compounds are widely distributed in plants and foods being regarded as significant antioxidants. The phenolic compounds exhibit several anti-aging, anti-inflammatory, anti-diabetic, anti-cancer and anti-angiogenic properties (Campos, Webby, Markham, Mitchell, & Da Cunha, 2003; Laaroussi et al., 2020).

The amounts of TPC and TFC of the BP extracts are shown in Fig. 2. In the present work, from the samples of Turkey the *Coronilla* sp. (BP8 TR) BP extract showed the highest TPC (41.17 mg GAE/g), followed by *Cistus* and *Pyrus* sp. (BP10 TR) (38.06 mg GAE/g), *Castanea* sp. (BP9 TR) (29.89 mg GAE/g) and Lamiaceae (BP4 TR) (28.20 mg GAE/g), whereas the Rosaceae (BP3 TR) BP extract showed the lowest level (16.40 mg GAE/g). Similarly, from the samples of Romania the Rosaceae (BP6 RO) BP extract showed the highest TPC (38.53 mg GAE/g), followed by *Brassica* sp. (BP4 RO) (38.53 mg GAE/g) and Rosaceae family (BP7 RO) (35.94 mg GAE/g). At the same time, the pollens from the Fagaceae family (*Castanea* sp.) (BP8 RO) showed the lowest level (17.96 mg GAE/g). The TFC ranged between 7.716 and 2.39 mg QE/g. The highest levels were found in Lamiaceae BP4 TR (7.16 mg QE/g), followed by Fagaceae (*Castanea* sp.) BP8 RO (4.79 mg QE/g). Similarly, the lowest levels were observed in BP2 RO Fabaceae (2.50 mg QE/g) and BP5 TR (multifloral) (2.39 mg QE/g), respectively.

The BP from Portugal, Spain, Turkey, USA, Brazil, China, Egypt, New Zealand, and Greece had TPC values ranging between 0.50 and 213 mg GAE/g and TFC values ranging between 1.00 and 17.50 mg QE/g, respectively (Araújo et al., 2017; Komosinska-Vassev et al., 2015; Pascoal, Rodrigues, Teixeira, Feás, & Estevinho, 2014; Ulusoy & Kolayli, 2014). Comparatively to our study, Margaoan et al. (2013) showed that the highest polyphenol concentration was determined in *Prunus* sp. (8.87 mg GAE/g), followed by *Malus domestica* Borkh. (7.74 mg GAE/g and *Salix* sp. BP (7.69 mg GAE/g). The lowest level of TPC was obtained for BP from *Calluna vulgaris* (L.) Hull (3.76 mg GAE/g). In the case of TFC the content ranged between 2.55 mg QE/g (*Calendula officinalis*) and 6.29 mg QE/g (*Malus domestica*). The TPC evaluated by Barbieri et al., 2020, was for *Prunus* sp. (18.98 GAE/g fw), and Brassicaceae (17.82 mg GAE/g). Regarding the TFC, the values ranged between 7.75 and 23.46 mg CE/g.

The differences in the phytochemical composition of the BP samples, particularly those belonging to the same plant, might depend not only on their botanical origin but also on other factors, such as beekeeping

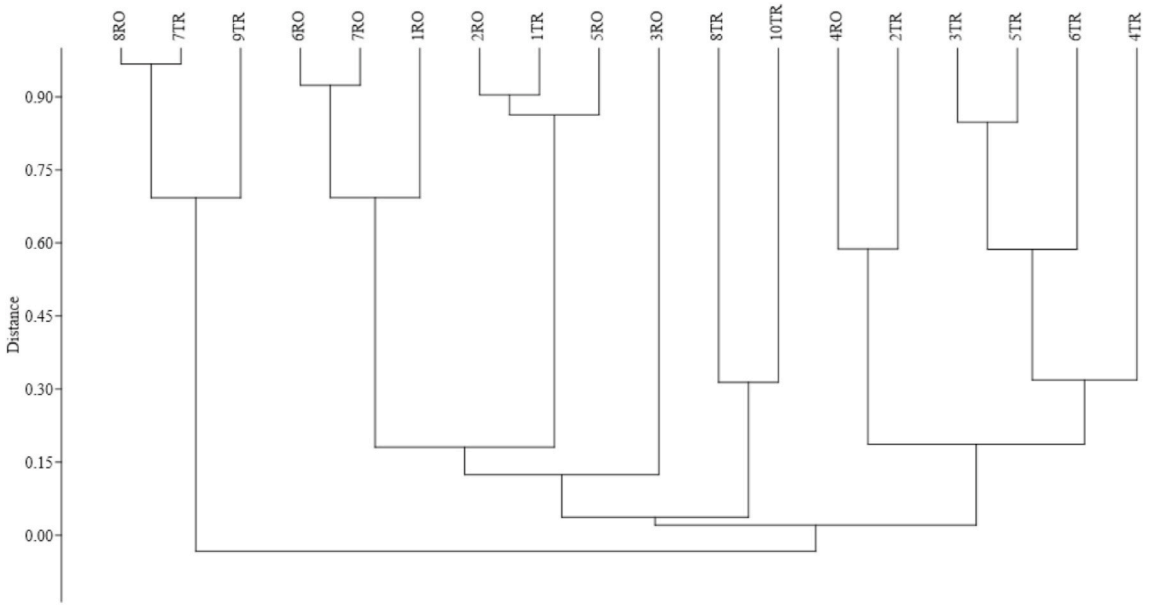

Fig. 1. Hierarchical clustering of the bee collected pollen samples based on palynological analysis (Euclidean distance, $r = 0.91$).

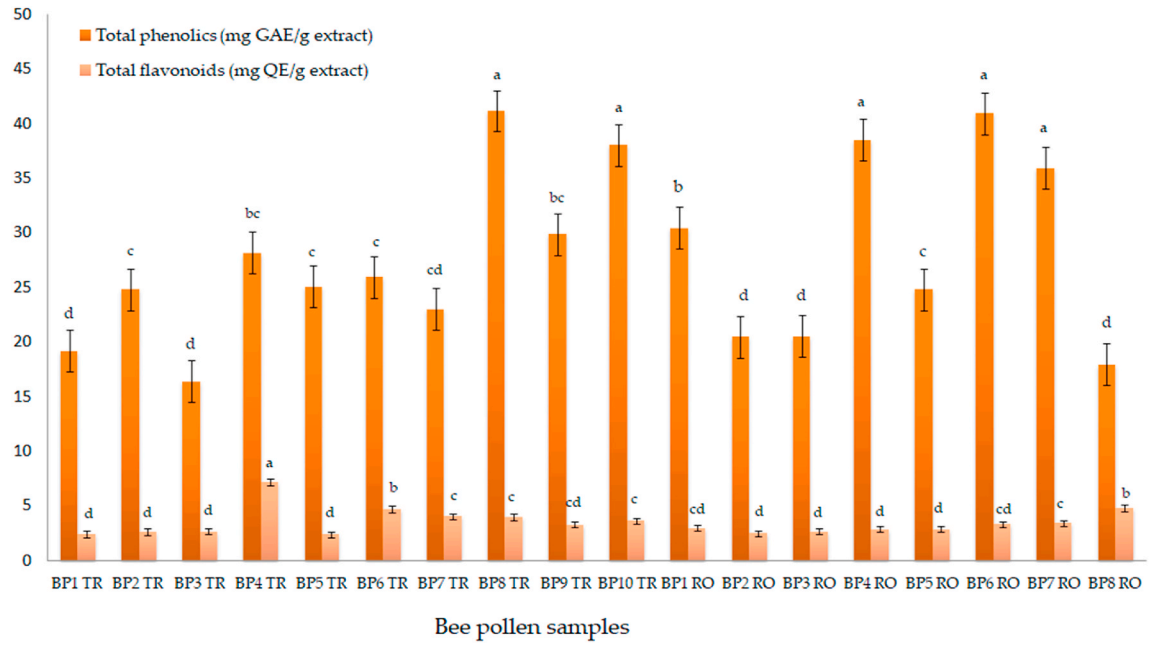

Fig. 2. Concentration of total phenolics and flavonoids of the bee collected pollen extracts (mean ± SD). Different letters represent significant differences ($p < 0.05$). Phenols are expressed as mg gallic acid equivalents/g extract (mg GAE/g extract); while flavonoids are expressed as mg quercetin equivalents/g extract (mg QE/g extract).

activity and climatic conditions. This aspect confirms the great variability in the chemical composition of the tested samples.

3.3. Antioxidant activity of the bee pollen extracts

Antioxidant activities of the BP samples were evaluated by a free radical scavenging assays (DPPH and TEAC). Generally, the values for DPPH ranged between 0.81 (BP8 RO) and 2.93 mmol Trolox/g (BP8 TR) and for TEAC the lowest value was 3.09 mmol Trolox/g (BP3 TR) and the highest 9.64 mmol Trolox/g (BP8 TR). Regarding the DPPH assay, the samples which exhibited the highest antioxidant activity were mostly multifloral and predominant in Fabaceae and Fagaceae, whereas for the TEAC assay the highest inhibitory activities were noticed in the samples predominant in Asteraceae, Brassicaceae, Fabaceae and Fagaceae. Mayda et al., 2020 showed that the DPPH values ranged between 3.08 and 3.85 mg Trolox/g in the BP samples. In a different study, Marghitas et al., 2009 demonstrated that the DPPH values of BP samples ranged between 0.13 (*Pinus* sp.) and 2.81 mmol Trolox/g (*Salix* sp.). For TEAC, the highest value was 6.838 mmol Trolox/g in *Salix* sp. and the lowest 0.546 mmol Trolox/g for *Pinus* sp. Ulusoy & Kolayli, 2013, evaluated the antioxidant activity of multiple BP samples from Turkey. Their results showed that for the TEAC the BP samples had values between 33.1 and 91.8 μmol Trolox/g. Furthermore, Margaoan et al., 2013 showed that the DPPH values ranged between 0.38 (*Calluna vulgaris*) and 1.65 (*Salix* sp.) mmol Trolox/g. Recently, Muñoz, Velásquez, Rodriguez, Montenegro, & Giordano, 2020 demonstrated that the antioxidant activity is strongly related to the pollen plant families. Thus, the *Brassica campestris* and *Galega officinalis* BP had significant DPPH

Table 1
The antioxidant activities, antibacterial and enzyme inhibitory activity of BPextracts.

	Antioxidant activity		Antibacterial activity			Enzyme inhibitory activity
Samples	DPPH (mmol Trolox/g)	TEAC (mmol Trolox/g)	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	α-AMY IC_{50} (mg/mL)
BP1 TR	1.40 ± 0.13f	3.46 ± 0.01e	2.05 ± 0.9ef	12.20 ± 0.7a	20.49 ± 2.9a	12.73 ± 0.16c
BP2 TR	1.35 ± 0.02f	3.54 ± 0.03e	2.64 ± 0.94d	8.46 ± 2.5b	16.66 ± 3.2b	11.25 ± 0.13de
BP3 TR	1.15 ± 0.02g	3.09 ± 0.04e	3.08 ± 0.05c	5.19 ± 1.7d	20.89 ± 3.8a	14.48 ± 0.21a
BP4 TR	1.37 ± 0.04 fg	7.60 ± 0.06bc	1.58 ± 0.04gh	5.25 ± 1.8d	5.48 ± 2.0de	10.22 ± 0.18e
BP5 TR	1.34 ± 0.06 fg	3.43 ± 0.02e	5.44 ± 2.01a	4.16 ± 1.8de	16.77 ± 4.1 ab	10.80 ± 0.22e
BP6 TR	0.98 ± 0.03i	4.19 ± 0.05de	4.3 ± 2.05 ab	4.22 ± 1.6de	10.39 ± 3.7c	10.58 ± 0.27e
BP7 TR	0.87 ± 0.04i,j	3.50 ± 0.01e	3.19 ± 0.13c	5.27 ± 2.0d	16.22 ± 2.3b	11.72 ± 0.30d
BP8 TR	2.93 ± 0.01a	9.64 ± 0.02a	3.92 ± 1.62 ab	8.96 ± 1.6b	10.36 ± 3.1c	8.13 ± 0.15f
BP9 TR	2.21 ± 0.01d	6.84 ± 0.02c	2.60 ± 0.91d	4.15 ± 1.8de	16.40 ± 2.3b	10.02 ± 0.13e
BP10 TR	2.39 ± 0.02cd	8.10 ± 0.32b	1.50 ± 0.07h	4.11 ± 1.9ef	7.26 ± 1.8d	8.52 ± 0.32f
BP1 RO	0.91 ± 0.01j	6.97 ± 0.06c	3.15 ± 0.07c	5.33 ± 1.9d	8.27 ± 2.5d	9.81 ± 0.25e
BP2 RO	1.05 ± 0.02h	4.46 ± 0.03d	5.1 ± 1.84a	12.45 ± 0.8a	10.61 ± 1.3c	12.58 ± 0.28c
BP3 RO	1.43 ± 0.02 fg	4.37 ± 0.04d	3.08 ± 0.05c	6.30 ± 2.24c	10.31 ± 2.6c	12.55 ± 0.37c
BP4 RO	2.77 ± 0.01bc	7.71 ± 0.01bc	1.63 ± 0.13g	5.84 ± 1.1d	5.08 ± 1.8ef	8.35 ± 0.19f
BP5 RO	1.24 ± 0.03 fg	4.43 ± 0.04d	2.0 ± 0.88ef	4.41 ± 2.0de	6.40 ± 0.6de	11.27 ± 0.24de
BP6 RO	2.84 ± 0.01b	9.33 ± 0.02a	1.53 ± 0.07gh	3.23 ± 0.6f	5.11 ± 2.1ef	8.10 ± 0.31f
BP7 RO	1.62 ± 0.17e	7.11 ± 0.03c	3.96 ± 1.55 ab	4.19 ± 1.9de	8.28 ± 1.7d	9.12 ± 0.11ef
BP8 RO	0.81 ± 0.03j	3.61 ± 0.03e	1.56 ± 0.11gh	3.11 ± 0.3f	4.20 ± 2.0f	13.41 ± 0.22b
Control						7.21 ± 0.21g
Acarbose						

Note: Within the same column, different letters (a–j) indicate significant differences ($p < 0.05$).

scavenging capacity compared to the other pollen types, with values from 0.98 to 4.74 mg Trolox/g.

3.4. Antibacterial activity of the bee pollen extracts

Antibacterial activity of the BP extracts was tested against the Gram-positive bacteria: *S. aureus* and the Gram-negative bacterium *P. aeruginosa* and *E. coli*. The results were expressed as mg/mL and are shown in Table 1. For the Gram-positive bacteria the MIC was noticed for BP10 TR (Asteraceae) with 1.50 mg/mL, followed by BP6 RO (Fabaceae) with 1.53 mg/mL. For the Gram-negative bacteria, the MIC values in all tested microorganisms were 3.11 mg/mL for samples BP8 RO (polyfloral) and 3.23 mg/mL for BP6 RO (Rosaceae). Our results are in accordance with Mohdaly, Mahmoud, Roby, Smetanska, & Ramadan, 2015 which showed that the Gram-positive bacteria were inhibited by BP extracts at low concentrations of 0.30 (mg/mL) for *L. monocytogenes*, and 0.78 (mg/mL) for *S. aureus*. Gram-negative bacteria (*E. coli* and *S. enterica*) proved to be sensitive to the BP extracts, having a MIC value of 1.25 and 1.35 mg/mL, respectively. Furthermore, Pascoal et al., 2014 showed that the most sensitive to the BP extracts proved to be *S. aureus* with MIC values between 1.81 (Boraginaceae) and 4.01 mg/mL (Cistaceae). Regarding *P. aeruginosa*, the MIC values were from 3.71 to 5.23 mg/mL in the BP sample dominant in Boraginaceae. Lastly, *E. coli* proved to be resistant to almost all tested extracts, except the Boraginaceae predominant extract, with values between 4.17 and 9.49 mg/mL.

3.5. Enzyme inhibitory activity

The BP extracts were also evaluated regarding the inhibitory activities of α-amylase (α-AMY) associated with Diabetes mellitus. The results were expressed as IC_{50} and are shown in Table 1. Extracts BP8 TR (Fabaceae) and BP10 TR (multifloral) had higher inhibition activities for (α-AMY) and did not differ statistically. Similarly, extracts BP6 RO and BP8 TR had relatively high inhibitory activities, 8.10 and 8.13 mg/mL, respectively. It can be observed that TPC content is related to the α-AMY, as well as the antioxidant activity. Regarding the Romanian samples, BP4 RO (multifloral), BP6 RO (Rosaceae) and BP7 RO (Rosaceae) also had strong inhibitory activities. These results are in accordance with Araújo et al. (2017), which found that *Eucalyptus* sp. pollen extracts exhibited the highest inhibitory activities of α-amylase, followed by multifloral extracts. Multiple studies reported the α-amylase inhibitory properties of BP (Araújo et al., 2017; Daudu, 2019; Keskin & Özkök, 2020).

3.6. Total lipids content (TLs)

TLs (g/100 g BP, dry mass) are rather diverse, with values ranging between 1—13 (Campos et al., 2008). The discrepancy mainly depends on pollen's type (Margaoan et al., 2014). The TLs contents in the BP samples are presented in Table 2. Within the analyzed samples, the highest levels of lipids were found in sample BP2 TR and BP3 TR with values of 9.06 and 8.56 g/100g lipids, respectively. Samples were followed by BP6 RO with a content of 7.91 g/100g lipids. Comparatively, sample BP7 TR presented the lowest value of 2.46 g/100g lipids. Mayda et al., 2020, reported lower values of lipid content in BP samples which varied between 2.59 and 5.12 g/100g.

3.7. Fatty acids composition

The fatty acid compositions, total ω-3, ω-6, polyunsaturated fatty acids (PUFAs) and the ω-6/ω-3 ratios of TLs from the BP samples from Romania and Turkey are summarized in Table 2. Fourteen fatty acids were identified in the pollen extracts from which the most abundant was α-linolenic [18:3 (ω-3)], with values ranging between 21.53% (BP7 RO) and 43.26% (BP2 TR). The next most abundant fatty acids were linoleic acid [18:2 (ω-6)] with values ranging between 13.54% (BP2 TR) to 38.29% (BP7 TR), followed by palmitic (16:0) with values from 16.25% (BP 9 TR) to 28.66% (BP1 RO), oleic [18:1 (ω-9)] with values between 2.96% (BP8 RO) and 14.88% (BP7 RO) and stearic (18:0) acids with values ranging from 2.06% (BP8 RO) to 5.75% (BP3 TR). Small quantities (<3%) of caproic (6:0); caprylic (8:0); capric (10:0); myristic (14:0); elaidic [18:1 (*9t*) ω-9], arachidic (20:0); 11-eicosenoic [20:1 (ω −9)] and behenic (22:0) acids were also identified. Regarding the ω-6/ω-3 (PUFAs ratio) the values ranged between 0.31 (BP2 TR) and 1.40 (BP7 RO), with an average value of 0.83, value close recommendations of other authors (ω-6/ω-3 = 1–5/1) as valuable to human health (Bastos et al., 2004; Conte et al., 2017).

Statistical analysis of the FAs classes showed significant differences ($p < 0.05$) between the analyzed BP samples. The highest saturated fatty acids (SFAs) ($p < 0.05$) values were recorded in BP3 TR (Asteraceae), followed by BP3 RO (Rosaceae *Filipendula* sp.). Samples BP7 TR (Fagaceae *Castanea* sp.), BP9 TR (Fabaceae *Coronilla* sp. and *Onobrychis* sp.) and BP4 TR (Lamiaceae) were the richest in unsaturated fatty acids (UFA) (>72%). Samples BP6 RO and BP7 RO predominant in Rosaceae, had the highest content in monounsaturated fatty acids (MUFAs), followed by the mainly polyfloral samples BP9 TR (Fagaceae *Castanea* sp., Lamiaceae *Teucrium* sp.) and BP10 TR (Cistaceae, Rosaceae *Pyrus* sp.). The highest values in PUFAs (>65%) were recorded for BP7 TR (Fagaceae *Castanea* sp.) and BP8 RO (Asteraceae, Fagaceae *Castanea* sp.).

The average value of UFAs (67.20%) exceeded that of SFAs (32.81%). A higher average value was also noticed for the PUFAs (57.83%) compared to MUFAs (9.37%). In their extensive review, Thakur & Nanda, 2020 highlighted that nearly 20 FAs are found in BP among which ω-3 fatty acids are dominating. Myristic, stearic and palmitic acids are detected as major SFAs, while α-linolenic, linoleic and oleic acids were the most prevalent UFAs. Previously, Thakur and Nanda (2018) reported the values of UFA to SFA ratio to range from 2.2 to 6.7 which relates to the BP lipid's high quality. It was demonstrated that a high value of UFA/SFA reduces the levels of fats and cholesterol, thus preventing cardiovascular disease, making BP a significant ω-3 FAs source for improving human health (Domínguez-Valhondo et al., 2011; Glick & Fischer, 2013; Simopoulos & DiNicolantonio, 2016; Thakur & Nanda, 2020). Furthermore, the UFAs are an important component of membrane phospholipids and helps maintain membrane fluidity, thus improving the membrane functionality and cell metabolism (Mărgăoan et al., 2014).

According to previous studies, FAs are similar in different pollens but their proportion varies according to the floral sources and geographical regions (Komosinska-Vassev et al., 2015). Recently it was also demonstrated that honeybees prefer the pollens which contain a higher amount of UFAs compared to SFAs and that bees have differential dance behaviour in order to accomplish colony's different nutritional needs of essential FAs (Zarchin, Dag, Salomon, Hendriksma, & Shafir, 2017). In our study, the amount of linoleic acid varied between 13.54% (BP2 TR) and 38.29% (BP7 TR). Comparatively, the linoleic acid ranged between 3.25 and 11.32 g/100 g in Indian BP, 2.66–24.38 g/100 g for the BP from China, whereas the BP from Romania had values between 7.62 and 33.21 g/100 g. Comparatively, the α-linolenic acid ranged between 0.5 and 16.28 g/100 g for Indian BP, 20.28–46.93 g/100 g in Romanian BP, and 4.11–58.52 g/100 g in Chinese BP (Mărgăoan et al., 2014; Thakur & Nanda, 2018; Yang et al., 2013). Mayda et al., 2020 detected a total of 22 different FAs, from which five (palmitic, stearic, oleic, linoleic and linoleic acids) were predominant in all BP samples. In small quantities, lauric, eicosenoic and heptadecanoic acids were also detected. Manning, 2001 also reported that there are distinct differences between plant species, such as *Helianthus annuus* (sunflower) pollen, which was dominant in myristic acid.

Different statistical methods were used in order to make different correlations between the BP samples and their composition. The dendrograms of the hierarchical clustering and heatmap results are shown in Fig. 3. According to the PCA, the botanical origin of the BP samples

Table 2
Fatty acid composition (expressed as g/100g of total fatty acids) of the analyzed BP samples.

Fatty acid	BP1 TR	BP2 TR	BP3 TR	BP4 TR	BP5 TR	BP6 TR	BP7 TR	BP8 TR	BP9 TR	BP10 TR	BP1 RO	BP2 RO	BP3 RO	BP4 RO	BP5 RO	BP6 RO	BP7 RO	BP8 RO
(6:0)	0.08 ± 0.01c	0.14 ± 0.02b	0.13 ± 0.01b	0.09 ± 0.01c	0.06 ± 0.02cd	0.04 ± 0.01e	0.08 ± 0.02c	0.07 ± 0.01c	0.89 ± 0.02a	0.04 ± 0.01e	0.06 ± 0.01cd	0.06 ± 0.01cd	0.05 ± 0.01e	0.07 ± 0.02cd	0.07 ± 0.01cd	0.03 ± 0.01e	0.05 ± 0.01e	0.05 ± 0.02e
(8:0)	0.18 ± 0.02de	0.23 ± 0.07d	1.05 ± 0.21a	0.80 ± 0.01b	0.06 ± 0.01f	0.46 ± 0.02c	0.1 ± 0.01g	0.23 ± 0.02d	0.92 ± 0.04a	0.09 ± 0.01	0.18 ± 0.02de	0.16 ± 0.02de	0.02 ± 0.01g	0.07 ± 0.01f	0.17 ± 0.02de	nd	0.05 ± 0.01f	0.07 ± 0.02f
(10:0)	0.89 ± 0.03b	0.18 ± 0.01g	1.20 ± 0.11a	0.24 ± 0.02f	0.14 ± 0.01g	0.54 ± 0.03d	0.36 ± 0.01e	0.44 ± 0.03d	0.96 ± 0.02b	0.10 ± 0.01gh	0.14 ± 0.01gh	0.62 ± 0.03c	1.17 ± 0.07a	0.16 ± 0.01g	0.51 ± 0.03d	0.03 ± 0.01i	0.14 ± 0.02gh	0.70 ± 0.03c
(12:0)	2.41 ± 0.11c	5.41 ± 0.21a	5.30 ± 0.21a	1.12 ± 0.04f	1.58 ± 0.02e	2.12 ± 0.03cd	0.18 ± 0.01g	1.66 ± 0.07e	2.80 ± 0.09b	1.08 ± 0.03f	1.47 ± 0.06e	1.81 ± 0.12cd	2.53 ± 0.11c	1.68 ± 0.03e	1.94 ± 0.06cd	0.57 ± 0.05g	2.46 ± 0.14c	1.16 ± 0.08f
(14:0)	2.08 ± 0.10b	1.02 ± 0.06e	0.66 ± 0.07f	0.72 ± 0.04f	1.32 ± 0.10cd	1.16 ± 0.12cd	0.58 ± 0.09	1.02 ± 0.07e	0.68 ± 0.06f	0.56 ± 0.09 fg	0.59 ± 0.05f	1.50 ± 0.05c	2.65 ± 0.04a	1.28 ± 0.12cd	1.33 ± 0.06cd	0.90 ± 0.02e	0.99 ± 0.01e	1.52 ± 0.03c
(16:0)	24.07 ± 0.30 ab	23.22 ± 0.28bc	22.7 ± 0.31c	19.20 ± 0.25	24.2 ± 0.21 ab	27.41 ± 0.31a	19.96 ± 0.21d	25.6 ± 0.26 ab	16.25 ± 0.17e	24.81 ± 0.23 ab	28.66 ± 0.30a	25.90 ± 0.27 ab	26.74 ± 0.31a	24.50 ± 0.32 ab	26.70 ± 0.22 ab	24.37 ± 0.25 ab	22.65 ± 0.19c	22.79 ± 0.29c
(18:0)	2.70 ± 0.03e	3.04 ± 0.11d	5.75 ± 0.12a	3.54 ± 0.13b	2.68 ± 0.12e	2.48 ± 0.10e	2.05 ± 0.09	2.47 ± 0.07e	2.43 ± 0.05e	2.43 ± 0.11e	2.15 ± 0.13ef	2.34 ± 0.21ef	2.12 ± 0.11ef	3.55 ± 0.09b	2.42 ± 0.14e	3.38 ± 0.13cd	3.78 ± 0.12b	2.06 ± 0.04efg
[18:1 (ω −9)]	6.70 ± 0.25ef	6.88 ± 0.21ef	8.69 ± 0.22d	7.82 ± 0.19e	8.76 ± 0.20d	7.38 ± 0.25e	8.21 ± 0.12d	7.54 ± 0.15e	10.87 ± 0.21b	10.45 ± 0.11b	6.18 ± 0.17g	6.31 ± 0.05g	5.62 ± 0.03h	9.67 ± 0.02c	6.44 ± 0.05g	14.36 ± 0.11a	14.88 ± 0.07a	2.96 ± 0.02i
[18:1 (*9t*) (ω −9)]	0.46 ± 0.02ef	0.55 ± 0.02e	0.51 ± 0.05e	0.42 ± 0.03efg	0.66 ± 0.02c	0.68 ± 0.08c	0.52 ± 0.03e	0.61 ± 0.05cd	0.48 ± 0.07ef	0.62 ± 0.03cd	0.70 ± 0.05c	1.75 ± 0.12a	0.51 ± 0.05e	0.46 ± 0.02ef	0.54 ± 0.06e	0.94 ± 0.04b	0.39 ± 0.02 fg	0.54 ± 0.06e
[18:2 (ω −6)]	22.80 ± 0.28ef	13.54 ± 0.19h	17.78 ± 0.25g	32.62 ± 0.31b	24.22 ± 0.20e	13.65 ± 0.11h	38.29 ± 0.29a	24.69 ± 0.12e	31.58 ± 0.14c	29.93 ± 0.23cd	21.96 ± 0.31	24.43 ± 0.13e	21.80 ± 0.11	23.06 ± 0.13ef	22.37 ± 0.28f	30.99 ± 0.14c	30.32 ± 0.31cd	32.94 ± 0.22b
[18:3 (ω −3)]	35.12 ± 0.45b	43.26 ± 0.29a	32.33 ± 0.11d	30.57 ± 0.28e	33.98 ± 0.13bc	42.07 ± 0.19a	28.64 ± 0.13f	33.52 ± 0.25bc	30.26 ± 0.14e	27.43 ± 0.35f	35.38 ± 0.22b	32.62 ± 0.17d	33.78 ± 0.65bc	32.84 ± 0.13d	34.82 ± 0.22b	22.56 ± 0.33g	21.53 ± 0.11h	33.21 ± 0.16cd
(20:0)	1.03 ± 0.02b	1.07 ± 0.02b	1.07 ± 0.01b	0.86 ± 0.02cd	0.88 ± 0.03cd	0.65 ± 0.01ef	0.14 ± 0.2g	0.7 ± 0.01e	0.84 ± 0.02cd	0.82 ± 0.03d	1.07 ± 0.01b	0.91 ± 0.02c	1.55 ± 0.03a	0.92 ± 0.02c	1.05 ± 0.02b	0.70 ± 0.03e	0.89 ± 0.01cd	0.74 ± 0.02e
[20:1 (ω −9)]	0.33 ± 0.01d	0.42 ± 0.3c	2.08 ± 0.02a	0.98 ± 0.03b	0.41 ± 0.01c	0.30 ± 0.01d	0.05 ± 0.01g	0.31 ± 0.02d	0.14 ± 0.01f	0.34 ± 0.01d	0.22 ± 0.02e	0.30 ± 0.01d	0.28 ± 0.02d	0.30 ± 0.03d	0.31 ± 0.01d	0.22 ± 0.01e	0.39 ± 0.02c	0.19 ± 0.01e
(22:0)	1.15 ± 0.12cd	1.04 ± 0.02e	0.75 ± 0.01g	1.02 ± 0.02e	1.05 ± 0.03e	1.06 ± 0.02e	0.84 ± 0.03	1.14 ± 0.02d	0.9 ± 0.01ef	1.34 ± 0.03b	1.24 ± 0.04c	1.29 ± 0.02b	1.18 ± 0.01c	1.44 ± 0.05a	1.33 ± 0.07b	0.95 ± 0.04ef	1.48 ± 0.03a	1.07 ± 0.03e
SFA	34.59	35.35	38.61	27.59	31.97	35.92	24.29	33.33	26.67	31.27	35.56	34.59	38.01	33.67	35.52	30.93	32.49	30.16
UFA	65.41	64.65	61.39	72.41	68.03	64.08	75.71	66.67	73.33	68.77	64.44	65.41	61.99	66.33	64.48	69.07	67.51	69.84
MUFA	7.49	7.85	11.28	9.22	9.83	8.36	8.78	8.46	11.49	11.41	7.10	8.36	6.41	10.43	7.29	15.52	15.66	3.69
PUFA	57.92	56.80	50.11	63.19	58.20	55.72	66.93	58.21	61.84	57.36	57.34	57.05	55.58	55.90	57.19	53.55	51.85	66.15
∑ω-3 PUFAs	35.12	43.26	32.33	30.57	33.98	42.07	28.64	33.52	30.26	27.43	35.38	32.62	33.78	32.84	34.82	22.56	21.53	33.21
∑ω-6 PUFAs	22.8	13.54	17.78	32.62	24.22	13.65	38.29	24.69	31.58	29.93	21.96	24.43	21.80	23.06	22.37	30.99	30.32	32.94
ω-6/ω-3	0.6492	0.313	0.55	1.067	0.71277	0.324	1.337	0.73658	1.043622	1.0911411	0.62069	0.748927	0.645352	0.70219	0.6424469	1.37367	1.408268	0.99187
TLs	7.3 ± 0.03d	9.06 ± 0.32a	8.56 ± 0.36b	5.63 ± 0.32 fg	6.98 ± 0.32e	7.3 ± 0.35d	2.46 ± 0.36h	6.66 ± 0.36f	4.87 ± 0.36g	5.71 ± 0.31 fg	6.93 ± 0.30e	6.91 ± 0.40e	6.19 ± 0.34f	7.01 ± 0.34e	7.12 ± 0.36de	7.91 ± 0.41c	5.95 ± 0.36f	5.00 ± 0.36g

PUFAs, polyunsaturated fatty acids; caproic acid (6:0); caprylic acid (8:0); capric acid (10:0); lauric acid (12:0); myristic acid (14:0); palmitic acid (16:0); stearic acid (18:0); oleic acid [18:1 (ω-9)]; elaidic acid [18:1(*9t*) ω-9]; linoleic acid [18:2 (ω-6)]; α-linolenic acid [18:3 (ω-3)]; arachidic acid (20:0); 11-eicosenoic acid [20:1(ω-9)]; behenic acid (22:0). The values represent the average of three independent determinations.

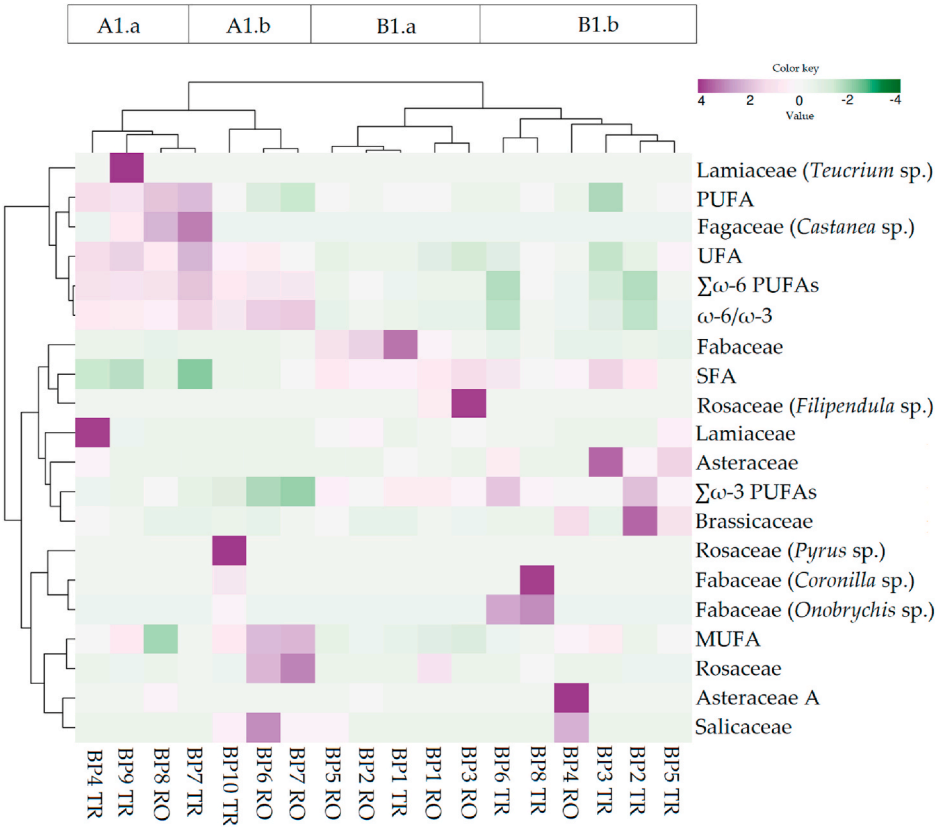

Fig. 3. Hierarchical clustering and heatmap visualization of the BP samples based on the fatty acids and palynological profiles. Columns indicate the BPsamples and rows the fatty acids and palynological analysis. Cells are coloured based on the concentration in each BP sample, where purple represents a positive correlation and green a negative correlation. The row dendrogram resulted from the correlation between the fatty acids and the predominant pollen content; the column dendrogram showed the correlation between the BPsamples from Romania and Turkey. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

was correlated with selected FAs based on their concentration. Thus, the 1st group (A1. a) comprises samples BP4 TR, 7 TR and 9 TR and 8 RO, followed by BP10 TR, 6 RO and 7 RO which clustered together in the 2nd group (A1. b). The first cluster comprises the samples with predominant (>45%) and secondary (16–45%) pollens from Fagaceae (*Castanea* sp.) and Lamiaceae families, which exhibited high concentrations in PUFAs and UFAs, as well as in linoleic acid. As it can be foreseen in the 2nd cluster (A1. b), samples BP5-7 RO exhibited high concentrations of MUFAs, as well as predominant pollen in Rosaceae and Salicaceae, respectively. Samples BP1-3 RO and 5 RO and sample BP1 TR, with Fabaceae and Rosaceae (*Filipendula* sp.) as predominant plant family and a significant content in SFAs, clustered in the 3rd group (B1. a). Comparatively, the 1st and 2nd clusters negatively correlated with SFAs and α-linolenic acid. The 4th cluster (B1. b) grouped samples BP2-6 TR, 8 TR and 4 RO (Asteraceae A and Salicaceae). Samples BP6 TR and 8 TR had Fabaceae (*Coronilla* sp. and *Onobrychis* sp.) as predominant plant families. All samples exhibited high α-linolenic acid content. Furthermore, a negative correlation was noticed for PUFA, MUFA and ω6 to ω3 ratio. Our results are in accordance with Kostić et al., 2017 who identified palmitic acid as the predominant FA to *Brassica* sp. in Serbian BP,

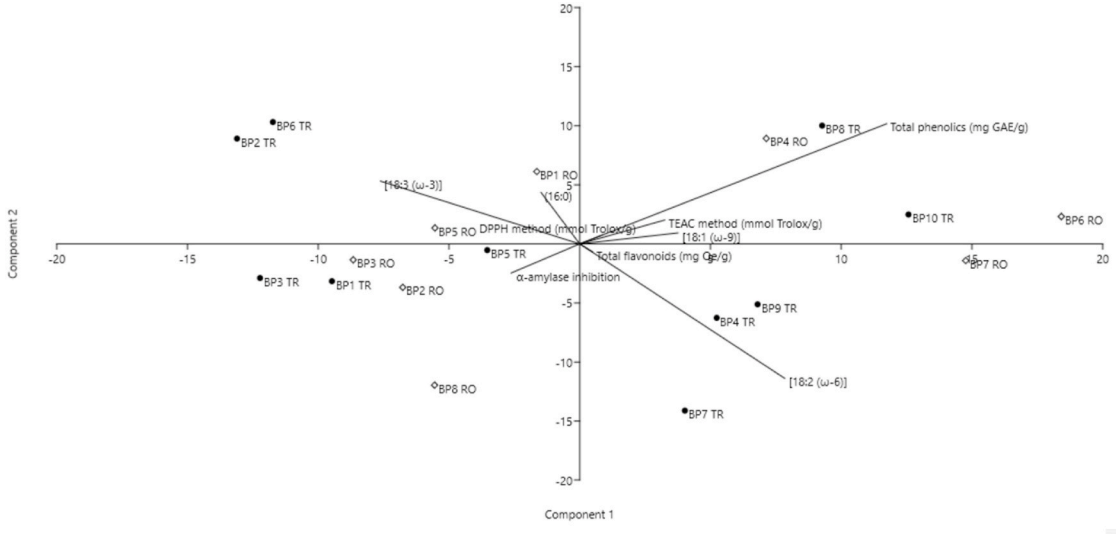

Fig. 4. Principal component analysis biplot obtained for the BP samples, selected fatty acids, total phenolics and flavonoids, antioxidant and enzyme inhibitory activities. The first two components explained 89% of the data variance.

being also a sign for its' antimicrobial activity. The *Brassica* pollen also had a significant content in linolenic and oleic acids, whereas the *Salix* pollen has high content in palmitic, stearic and linoleic acids. According to their results, a strong correlation was noticed for the FAs and palynological analysis. Furthermore, Conte et al. (2017) demonstrated that there is a strong correlation between the FAs composition and botanical origin of Brazillian BP.

On the basis of the PCA, the BP samples were not clearly discriminated by using all the data. Thus, introducing only the TPC, TFC, selected FAs, and antioxidant and enzyme inhibitory activities a good differentiation was obtained (the first two principal components explaining 89% of the data variance). As shown in Fig. 4, the TPC, α-linolenic and linolenic acids clearly discriminated the BP samples and to a lesser extent by the other parameters used. According to the biplot, in the 1st quadrant samples BP1 TR, 3 TR and 5 TR and BP2 RO, 3 RO and 8 RO had the highest values and at the same time exhibited the lowest inhibitory activities. In the 2nd quadrant, samples BP4 TR, 7 TR and 9 TR exhibited the highest linoleic acid content. The 3rd quadrant highlights samples BP8 TR and 10 TR and samples BP4 RO, 6 RO and 7 RO with the highest TPC, as well as high concentrations in oleic acid. In the last quadrant samples BP1 RO and 5 RO have strong antioxidant activities and high concentrations of palmitic and α-linolenic acids. Thus, the antioxidant activity showed by BP samples can be attributed to their TPC and TFC. Our results are in accordance with Velásquez et al. (2017) which demonstrated that there is a strong correlation between the TPC and the antioxidant activity. Furthermore, our study also demonstrates that FAs content are strongly correlated to the BP plant families, as well as with their antioxidant and enzyme inhibitory activities.

We performed a second PCA in order to emphasise if there are significant correlations between the FAs and antibacterial activity, but the BP samples were not clearly discriminated by using all the FAs data. Thus, introducing only the antibacterial activity and palmitic, linoleic and α-linolenic acids as variables in the PCA, a good discrimination of the BP samples was achieved, the first and second components explaining 84% of the total variance (Fig. 5). In the 1st quadrant samples BP4 TR and 10 TR, as well as BP6-8 RO had the highest antibacterial activity against the tested Gram-positive and negative bacteria. The samples from Turkey had relatively high α-linolenic acid concentrations, whereas the samples from Romania were rich in linoleic acid. In the 2nd quadrant samples BP6 TR and 8 TR and BP1-5 RO had moderate to low antibacterial activity, but significant α-linolenic and palmitic acids. The 3rd and 4th quadrant grouped the samples from Turkey (BP1-3, 5, 7 and 9) which exhibited high antibacterial activity against *S. aureus* and *P. aeruginosa* and moderate to low against *E. coli*. Comparatively, Soares de Arruda et al., 2020, study observed moderate and weak correlations between the TPC, TFC, antioxidant, linoleic acid and antibacterial activity. In a different aspect, Velásquez et al., 2003 demonstrated that the botanical origin strongly influences the positive and negative correlations between the palynological analysis and antibacterial activity, as there is a positive correlation between the antibacterial activity of BP samples against *S. aureus* and *Medicago sativa* and a negative correlation with Malvaceae and Myrtaceae families. Consistent with our results, Manning, 2001 and Feldlaufer, Knox, Lusby, & Shimanuki, 1993 stated that linoleic and linolenic acids have strong antimicrobial properties against the honey bee pathogen *Paenibacillus larvae*, *Ascosphaera apis* (chalkbrood), *Melissococcus pluton*, as well as against *Bacillus cereus*, *B. alvei*, *B. laterosporus* and *Enterococcus faecalis*. In a different aspect, FAs such as palmitic, stearic and oleic were antimicrobial inactive.

4. Conclusions

In the present study, we showed that bee pollen is a rich source of polyphenols and fatty acids, displaying good antioxidant and enzyme inhibitory activities, which depend on the botanical origin. Bee pollen is a complex matrix that differs greatly in composition and is closely correlated with is botanical origin, collection and storage. The fatty acids content of the bee pollen samples is strongly associated with their phenolics content and moderately associated with flavonoids and antioxidant activity. Furthermore, the samples from Romania were dominant in α-linolenic, linolenic and palmitic acids and with higher phenolics content were strongly correlated to the antimicrobial activity. Comparatively, the samples from Romania had slightly higher values in myristic and behenic acids, whereas the samples from Turkey were higher in lauric and stearic acids. Additional attention should be paid to the fatty acids found in minor concentrations (i.e. capric, lauric and myristic acids) as studies reported their significant antimicrobial activities. Further analysis on monofloral pollen is required to evaluate their antioxidant and antimicrobial activities based on specific compounds (i.e. fatty acids), as linoleic and linolenic were present in high concentrations. The obtained results suggest that bee pollen samples tested in the present study could be sources of potential value-added nutrients

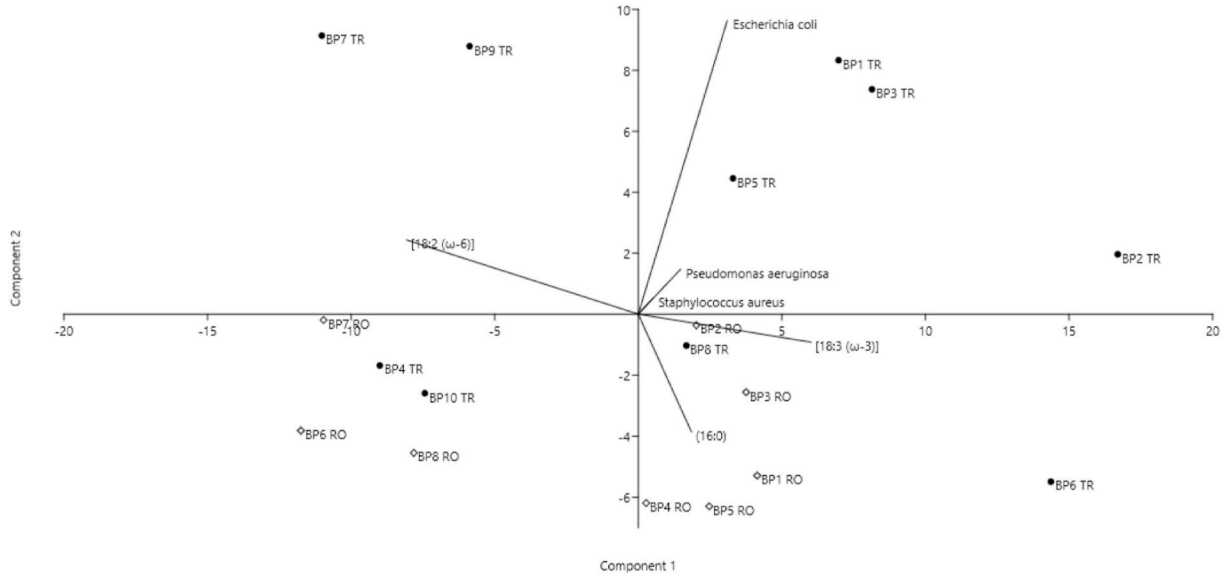

Fig. 5. Principal component analysis biplot obtained for the BP samples, selected fatty acids and antibacterial activity. The first two components explained 84% of the data variance.

and bioactive compounds. Furthermore, additional data regarding the pollen flora collected and stored by honeybees in these countries are provided.

CRediT authorship contribution statement

Rodica Mărgăoan: Conceptualization, Supervision, Methodology, Resources, Data curation, Writing – original draft, Writing – review & editing. **Aslı Özkök:** Conceptualization, Methodology, Supervision, Methodology, Data curation, Writing – original draft. **Şaban Keskin:** Methodology, Investigation. **Nazlı Mayda:** Methodology, Investigation, Writing – review & editing. **Adriana Cristina Urcan:** Methodology, Investigation, Visualization. **Mihaiela Cornea-Cipcigan:** Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.111925.

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