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MICROBIOLOGICAL QUALITY, ANTIOXIDATIVE AND ANTIMICROBIAL PROPERTIES OF SLOVENIAN BEE POLLEN

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ABSTRACT

Bee pollen can be considered as perfect food with a great nutritional value, high protein and essential amino acid content, vitamins and minerals. It can be a source of healthy nutrients, but as an animal product also of harmful microbial contaminants. The aim of our study was to determine potential health risks and benefits of Slovenian bee pollen. We determined its i) microbiological burden: aerobic mesophilic microorganisms, yeast, molds, and coliform bacteria in CFU/g; ii) polyphenolic content: the Folin-Ciocalteu method (mgGA/g); iii) antioxidative potential (AOP): $DPPH^{\bullet}$ scavenging assay (EC50 in mgGA/L); and iv) antimicrobial activity (MIC): microdilution method on *Escherichia coli*, *Listeria monocytogenes* and *Campylobacter jejuni*. We analyzed 14 samples of bee pollen gathered from 7 Slovenian geographical regions, from April until May 2017. The microbiological burden was high, with all indicator tests reaching up to 6.78 $\log_{10}$CFU/g of bee pollen, but the number of coliform bacteria in all samples from 2.00 to 4.48 $\log_{10}$CFU/g. The polyphenolic content and AOP of the samples was good, with up to 13.1 mg GA/g and as low as 2.4 mgGA/L (EC50), respectively. Interestingly, antimicrobial activity was not always in correlation with polyphenolic content, but always strongly against *E. coli*, substantial against *C. jejuni*, and negligible against *L. monocytogenes.* Our results show a great health potential of bee pollen for human health, but also the need of bee pollen processing improvement for its standardized quality and safety.

Key words: *Bee pollen, polyphenols, antioxidant, antimicrobial activity, microbiological safety.*

INTRODUCTION

Pollen is a microscopic structure produced in the anthers of plants and typical for every single botanical type. It presents the plants male reproductive organs that are the basis for sexual reproduction in plants. Pollination of plants is carried out by the wind or by insects, where bees play an important role. In the process of pollination when a bee touches the anthers its body becomes covered with pollen

dust. They gather this pollen dust, moisturize it with saliva and nectar, compress it into two pollen baskets on their hind legs, bring it to the beehive and store it in combs for their needs (Almeida Muradian et al., 2005; Bogdanov, 2012; Kieliszek et al., 2018).

Bee pollen can be considered as perfect food with great nutritional value. For human consumption beekeepers collect bee pollen using bee pollen traps placed at the entrance or on the bottom of the bee hive (Lilek et al., 2015). The presence of proteins, essential amino acids, carbohydrates, saturated and unsaturated fatty acids, dietary fibers, vitamins and minerals makes bee pollen very useful in human nutrition - in supplementary and alternative diets with functional and therapeutic properties. It has health promoting effects: anti-inflammatory, antimicrobial, antifungal, antioxidant and antitumor activities (Almeida-Muradian et al., 2005; Campos et al., 2010; Münstedt and Bogdanov, 2009; Bogdanov 2012; Kieliszek et al., 2018), with increasing attention among consumers.

Important functional ingredients of plant food are antioxidants. Bee pollen shows high contents of polyphenolic substances, mainly flavonoids and phenolic acids with potential antioxidant activity and thus preventive in cancerous diseases, cardiovascular diseases, inflammatory processes, neurological disorders and aging (Gómez-Caravaca et al., 2006; Campos et al., 2008; Kieliszek et al., 2018).

To achieve microbiological stability, beekeepers dry bee pollen under controlled conditions. This process will decrease the water activity of bee pollen from 0.7 to 0.3 and thus increase the stability of the product. Chemical, biological and sensory properties of bee pollen are better preserved if bee pollen is consumed fresh. Thus are the high hygienic standards and proper handling of the product in every stage of production crucial for its quality and safety. Opposite activities can cause the product becoming undesirable and harmful for human health (Deveza et al., 2015; Beev et al., 2018; Kieliszek et al., 2018).

In this investigation we examined i) the microbiological quality of bee pollen by determining the total aerobic count, yeast and mold count, and coliform bacteria, ii) the total phenolic content, ii) antioxidative potential (AOP), and iii) antimicrobial activity of bee pollen gathered from 7 different Slovenian statistical (geographical) regions.

MATERIALS AND METHODS

Samples collection: Fourteen samples of bee pollen from Carnolian bees (*A. mellifera carnica*) were collected in beekeeping season of 2017 from seven different geographical regions in period from April until May - from regions Goriška (n=1), Zasavska (n=1), Obalnokraška (n=1), Gorenjska (n=3), South-East (n=2), Pomurska (n=1) and Central (n=5), and refrigerated until analysis. For the microbiological analyses whole bee pollen grains were used, prior to chemical analyses bee pollen pallets were ground.

Microbiological analysis: Bee pollen samples were prepared and diluted in saline solution according to standard procedures (ISO 6887-1:1999). The total aerobic count was enumerated on PC agar (Merck, Germany), coliforms on VRBL agar

(Biolife, Italy), and molds and yeast on DRBC agar (Biolife, Italy). All samples were analyzed in triplicates and results are presented as the $\log_{10}$ number of colony forming units in 1 gram of bee pollen (CFU/g).

Preparation of bee pollen extracts: The pollen extracts were prepared by solid/liquid extraction using 96% ethanol as a solvent (1g/3mL), for 5 h on a shaker in the dark at room temperature with intermediate treatments in the ultrasonic bath. Further the samples were filtered and centrifuged for 10 min at 4000 rpm. At least three extracts for each pollen sample were obtained. The repeatability of extraction of phenolic compounds was within 5%.

Total phenolic content and antioxidative potential determination: The total phenolic compounds were determined according to the Folin-Ciocalteu method (Gutfinger, 1981) according to Terpinc et al. (2012). The content of total phenolic compounds in pollen samples was expressed as mg gallic acid (GA, Sigma Aldrich, Germany) per gram of dry weight of pollen (mg GA/g). All analyses were carried out in at least three repetitions. Determination error was less than 3%. The results for the content of phenolic compounds in pollen samples are given as the average value ± standard deviation.

The AOP of extracts of phenolic compounds from pollen was determined by the method of determining the effectiveness of scavenging radical $DPPH^{\bullet}$ (Brand-Williams et al., 1995) according to Terpinc et al. (2012). The analysis was done in 3 to 5 repetitions. AOP was expressed as the concentration of phenolic compounds in the reaction mixture, which reduced the initial $DPPH^{\bullet}$ content by 50% (EC50). A lower EC50 value means a better AOP.

Bacterial culture preparation: Cultures were grown on Mueller Hinton Agar (MHA, Biolife, Italy), *E. coli* and *L. monocytogenes* for 24 h at 37 °C aerobically, and *C.jejuni* at 42°C microaerobically (5% O_2, 10% CO_2 in N_2). Overnight cultures were prepared in Mueller Hinton broth (MHB, Sigma Aldrich, Germany). For antimicrobial activity testing the optical density at 600 nm (OD_{600}) of the overnight cultures was adjusted to 0.1 in MHB. Culture suspensions were further diluted to the concentration of $5x10^5$ CFU/mL.

Antimicrobial activity of bee pollen extracts: Extract of phenolic compounds (4 mL) was dried prior to antimicrobial testing. The solvent was evaporated in a centrifugal vacuum evaporator HT-4 series II (GeneVac Technologies). Dry residue was dissolved in 0.3 mL of dimethyl sulfoxide (DMSO, Sigma, Germany) and further in MHB. The MIC was determined for *E. coli* and *L.monocytogenes* according to Klančnik et al. (2009) and for *C. jejuni* according to Kovač et al. (2015). MIC is presented as mg of dried bee pollen extract per mL of solution (mg/mL).

Statistical analysis: The statistical analysis was carried out in the SPSS software version 21 (IBM Corp., Armonk, NY, USA). The correlation analysis was determined using the Pearson correlation coefficient. The statistical significance of the phenolic content, radical scavenging and antimicrobial action was determined using one-way ANOVA.

RESULTS AND DISCUSSION

Microbiological quality of bee pollen

Bee pollen is an animal product, gathered by bees and therefore exposed to conditions that enable a high microbial contamination. Although the composition of bee pollen is defined by the origin and mixture of plants pollinated by bees and can thus be very variable, this was not reflected in the microbiological variability of samples. The total aerobic count and the number of coliform bacteria were similar in all samples. In general, the microbiological load of samples from different geographical regions (Fig. 1) was similar. Only the Obalnokraška region stands out with the lowest average number of molds (<1 $\log_{10}$CFU/g) and the highest average number of yeast (7.59 $\log_{10}$CFU/g).

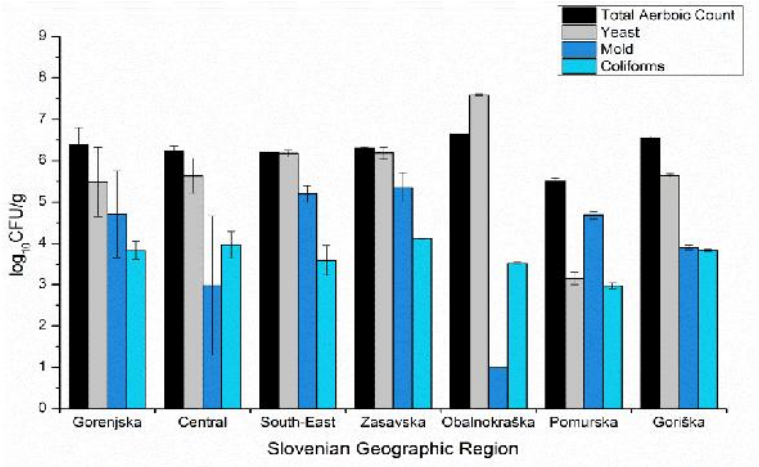

Figure 1. Total aerobic count, yeasts, molds, and coliforms in samples of fresh Slovenian bee pollen from 7 geographical regions, presented as the average $\log_{10}$CFU/mL ± standard deviation of all samples from one region. Samples with a value of <1 $\log_{10}$CFU/g are presented as 1 $\log_{10}$CFU/g in the graph without standard deviations.

The high microbial contamination of the fresh bee pollen samples in this study is similar to the samples observed by Mauriello et al. (2017) and Beev et al. (2018). Beev et al. (2018) reported water activity of fresh bee pollen of 0.7 and more, which enables the proliferation of yeast and molds. Bee pollen can thus be a subject of spoilage by these microorganisms or a vector for pathogenic fungi. Although this and lower water activity would not enable most pathogenic bacteria to proliferate, it can be a vector for the transfer of pathogenic bacteria (Sancho-Madriz, 2003; Beuchat et al., 2013). Coliform bacteria are usually considered an indication of fecal contamination and unsanitary conditions in food production, but it is also a diverse group of microorganisms that can be found in the natural environment (Martin et al., 2016). In a fresh food product such as bee pollen the presence of coliforms should be interpreted carefully as it cannot be considered as proof of either the presence of fecal pathogens or unsanitary conditions.

Phenolic content and antioxidative potential of bee pollen extracts

Results show that among samples there are notable differences ($p<0.05$) in the content of phenolic compounds (Table 2, Fig. S1). The content of phenolic compounds in investigated bee pollen ranged from 6.5 to 13.1 mg GA/g (Fig. S1). The highest phenolic content was observed in samples C2 (Central region 2) and P (Pomurska region), which were significantly higher compared to other samples ($p<0.05$). The lowest phenolic content was observed in the sample C5 (Central region 5). No correlation was found between the phenolic content and the location of bee pollen collection, although more sampling is needed for the confirmation of this observation. The content of total phenolic compounds in the investigated pollen of Slovenian origin coincides with the literature values for pollen originating from Brazil as reported by Carpes et al., (2007) of 7 mg GA/g in extract obtained by 90% ethanol. Our results are also in accordance with the values for the Portuguese bee pollen with total phenolic content ranging from 13 to 20 mg GA/g as reported by Feas et al. (2012) and with results of Morais et al. (2011) who obtained values from 10 to 17 mg GA/g. On the other side Pascoal et al. (2014) who prepared their extracts with methanol determined somewhat higher total phenolic content in bee pollen from Spain ranging from 18 to 29 mg GA/g. The differences can be explained by different solvents used for phenolic compounds extraction from bee pollen.

Table 2. Average, median, minimum and maximum content of total phenolic compounds expressed as mg gallic acid per gram of dry matter of pollen (mg GA/g) and antioxidative potential expressed as the concentration of phenolic compounds in the reaction mixture which reduced the initial $DPPH^{\bullet}$ content by 50% (EC50) for 14 samples.

	Content of total phenolic compounds (mg GA/g)	EC50 (mg GA/L)
Average	9.9	10.7
Median	9.8	10.6
Min	6.5	2.4
Max	13.1	22
No. of samples	14	14

The obtained results show that all investigated bee pollen extracts expressed the $DPPH^{\bullet}$ radical scavenging activity. However, the EC50 values considerably deviate between 14 analyzed samples (Fig. S2). The Pomurska region sample (P) expressed the highest AOP (EC50 = 2.4 mg GA/L; $p<0.05$) while the AOP of the Zasavska region sample was the poorest (EC50 = 22 mg GA/L) and differed significantly from all other samples ($p<0.05$). Campos et al. (2003) in their investigation determined appreciably lower AOP with EC50 ranging from 40 to 330 mg/L. The values for AOP of phenolic compounds in the investigated pollen samples of Slovenian origin are in accordance with the values of AOP, which were

determined in our previous research for phenolic compounds from propolis (Mavri et al., 2012), oil seeds (Terpinc et al., 2012), rosemary (Klančnik et al., 2009) and the EC50 value for the synthetic antioxidant BHT.

Antimicrobial activity of bee pollen extracts

The antimicrobial activity of bee pollen extracts was tested on two gram negative bacteria, *E. coli* and *C. jejuni*, and one gram positive bacterium, *L. monocytogenes*. The extracts showed no antimicrobial activity against *L. monocytogenes*. The antimicrobial activity of most samples (Table S2) was substantial against *E. coli* and *C. jejuni*. Samples G1 (Gorenjska region 1), C2 (Central region 2), P (Pomurska region), and GO (Goriška region) showed a better antimicrobial activity against *E. coli*, compared to other samples ($p<0.01$). Samples P and C2 also showed the highest antimicrobial activity against *C. jejuni* compared to other samples ($p<0.01$). A significantly higher antimicrobial activity ($p<0.01$) against *C. jejuni* was observed also in samples C1 (Central region 1) and SE1 (South-East region 1).

Table 3. Average, median, minimum and maximum antimicrobial activity of bee pollen extracts presented as the MIC in mg of dry extract/ mL, tested on *E. coli, C. jejuni* and *L. monocytogenes* from 14 samples.

	Minimal inhibitory concentration (MIC) in mg/mL		
	E. coli	*C. jejuni*	*L. monocytogenes*
Average	2.68	9.93	>6.25
Median	3.13	12.50	-
Min	1.56	0.78	-
Max	3.13	12.50	-
No. of samples	14	14	14

The antimicrobial activity of the bee pollen extracts against *E. coli* and *C. jejuni* showed a significant correlation to the total phenolic content of the extracts. When the total phenolic content was higher, the MIC was lower for *E. coli* ($r= - 0.634$, $p=0.015$) and *C. jejuni* ($r= - 0.537$, $p=0.048$). Extracts from bee products such as honey, propolis and bee pollen with high polyphenolic content have shown good antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* and other organisms (Grange and Davey, 1990; Estevinho et al., 2008; Mavri et al., 2012). The topic of antimicrobial activity of bee pollen is not widely researched, and the information available reports antimicrobial activity with the agar diffusion method rather than the microdilution method, making the comparison of results a challenge. Fatrcová-Šramková et al., (2016) and Morais et al. (2011) reported an antimicrobial activity against *E. coli* comparable to ours, but a better activity against Gram positive bacteria than Gram negative, although our results show the opposite.

SUPPLEMENTARY INFORMATION

Table S1. Microbiological contamination of fresh Slovenian bee pollen from 7 statistical regions presented with the $\log_{10}$CFU/g average ± standard deviation of total aerobic count, yeast, mold, and coliforms.

Sample designation	Statistical region	Average total count in $\log_{10}$CFU/g ± standard deviation			
		Total Aerobic Count	Yeast	Mold	Coliform bacteria
G1	Gorenjska	6.65 ± 0.03	5.98 ± 0.02	5.50 ± 0.20	3.52 ± 0.12
G2	Gorenjska	6.70 ± 0.00	4.30 ± 0.10	3.22 ± 0.22	3.95 ± 0.01
G3	Gorenjska	5.80 ± 0.02	6.18 ± 0.10	5.39 ± 0.39	4.01 ± 0.01
C1	Central	6.03 ± 0.03	6.17 ± 0.06	5.15 ± 0.15	3.63 ± 0.05
C2	Central	6.33 ± 0.02	5.46 ± 0.02	3.80 ± 0.20	4.07 ± 0.05
C3	Central	6.12 ± 0.48	4.92 ± 0.02	3.94 ± 0.10	4.52 ± 0.02
C4	Central	6.28 ± 0.02	5.84 ± 0.06	<1.00 ± - *	3.62 ± 0.15
C5	Central	6.38 ± 0.01	5.77 ± 0.07	<1.00 ± - *	3.97 ± 0.01
SE1	South-East	6.21 ± 0.06	6.10 ± 0.02	5.39 ± 0.09	3.95 ± 0.09
SE2	South-East	6.18 ± 0.02	6.26 ± 0.02	5.00 ± 0.30	3.23 ± 0.03
Z	Zasavska	6.31 ± 0.03	6.18 ± 0.14	5.35 ± 0.35	4.11 ± 0.01
OK	Obalnokraška	6.65 ± 0.06	7.59 ± 0.02	<1.00 ± - *	3.52 ± 0.04
P	Pomurska	5.51 ± 0.01	3.15 ± 0.15	4.68 ± 0.09	2.97 ± 0.07
GO	Goriška	6.55 ± 0.04	5.64 ± 0.04	3.90 ± 0.05	3.83 ± 0.02
-	All	6.26 ± 0.32	5.68 ± 1.00	3.88 ± 1.65	3.78 ± 0.38

*No standard deviation is presented as all tested samples had less than 1 $\log_{10}$CFU/g.

Table S2. Antimicrobial activity of bee pollen extracts presented as mg of dry extract/ mL, tested on *E. coli, C. jejuni* and *L. monocytogenes* of 14 samples from 7 geographical regions.

Sample designation	Minimal inhibitory concentration (MIC) in mg/mL		
	E. coli	*C. jejuni*	*L. monocytogenes*
G1	1.56	12.50	>6.25
G2	3.13	12.50	>6.25
G3	3.13	12.50	>6.25
C1	3.13	6.25	>6.25
C2	1.56	0.78	>6.25
C3	3.13	12.50	>6.25
C4	3.13	12.50	>6.25
C5	3.13	12.50	>6.25

SE1	3.13	6.25	>6.25
SE2	3.13	12.50	>6.25
Z	3.13	12.50	>6.25
OK	3.13	12.50	>6.25
P	1.56	0.78	>6.25
GO	1.56	12.50	>6.25

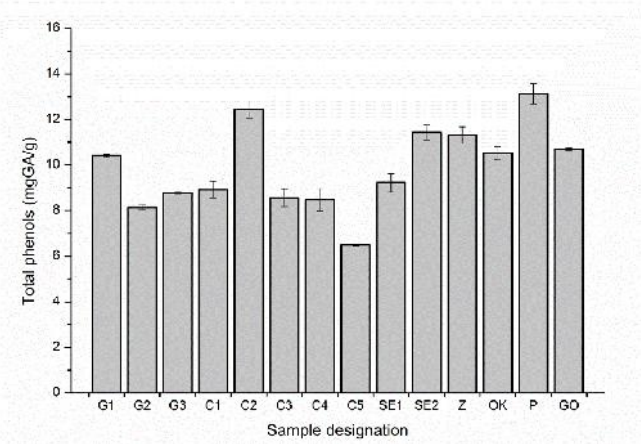

Figure S1. The content of total phenolic compounds in Slovenian bee pollen expressed as mg gallic acid per gram of dry matter of pollen (mg GA/g).

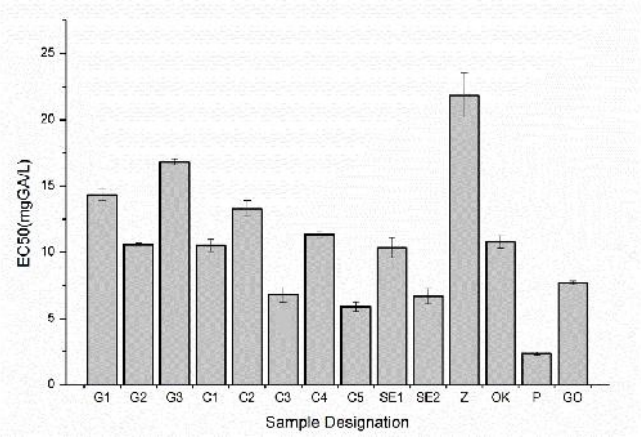

Figure S2. The antioxidative potential of Slovenian bee pollen expressed as the concentration of phenolic compounds in the reaction mixture which reduced the initial DPPH$^{\bullet}$ content by 50% (EC50).

CONCLUSION

The high microbiological burden of fresh bee pollen raises concerns about the suitability of bee pollen as a fresh product. It highlights the need for improvement

of bee pollen production and demands, alongside with a good manufacturing practice, additional steps to decrease the contamination. Despite the challenges in bee pollen production it has great potential as a nutritional supplement with an anti-oxidative effect and a high polyphenolic content that translates into good antimicrobial effect against enteric pathogenic bacteria, although this effect may vary between samples collected from different regions. These bioactivities together with a nutritional benefit make bee pollen a valuable product.

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REFERENCES

Almeida-Muradian, L.B., Pamplona, L.C., Coimbra, S., Barth, O.M. (2005). Chemical composition and botanical evaluation of dried bee pollen pellets. Journal of Food Composition and Analysis, 18, 105–111.

Beev, G., Stratev, D., Vashin, I., Pavlov, D., Dinkov, D. (2018). Quality assessment of bee pollen: a cross sectional survey in Bulgaria. Journal of Food Quality and Hazards Control, 5, 11–16.

Beuchat, L.R., Komitopoulou, E., Beckers, H., Betts, R.P., Bourdichon, F., Fanning, S., Joosten, H.M., Ter Kuile, B.H. (2013). Low-water activity foods: increased concern as vehicles of foodborne pathogens. Journal of Food Protection, 76, 150–172.

Bogdanov, S. (2012). Collection, Harvest, Composition and Quality - [WWW Document]. http://www.bee-hexagon.net/pollen/collection-harvest-composition-quality/ (Accessed 6.26.18).

Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28, 25–30.

Campos, M.G., Webby, R.F., Markham, K.R., Mitchell, K.A., Da Cunha, A.P. (2003). Age-induced diminution of free radical scavenging capacity in bee pollens and the contribution of constituent flavonoids. Journal of Agricultural and Food Chemistry, 51, 742–745.

Campos, M.G.R., Bogdanov, S., Almeida-Muradian, L.B. de, Szczesna, T., Mancebo, Y., Frigerio, C., Ferreira, F. (2008). Pollen composition and standardisation of analytical methods. Journal of Apicultural Research 47, 154–161.

Campos, M.G.R., Frigerio, C., Lopes, J., Bogdanov, S. (2010). What is the future of bee-pollen?. Journal of Apiproduct and Apimedical Science, 2, 131–144.

Carpes, S.T., Begnini, R., Alencar, S.M. de, Masson, M.L. (2007). Study of preparations of bee pollen extracts, antioxidant and antibacterial activity. Ciência e Agrotecnologia, 31, 1818–1825.

Deveza, M.V., Keller, K.M., Lorenzon, M.C.A., Nunes, L.M.T., Sales, É.O., Barth, O.M. (2015). Mycotoxicological and palynological profiles of commercial brands of dried bee pollen. Brazilian Journal of Microbiology, 46, 1171–1176.

Estevinho, L., Pereira, A.P., Moreira, L., Dias, L.G., Pereira, E. (2008). Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food and Chemical Toxicology, 46, 3774–3779.

Fatrcová-Šramková, K., Nôžková, J., Máriássyová, M., Kačániová, M. (2016). Biologically active antimicrobial and antioxidant substances in the *Helianthus annuus* L. bee pollen. Journal of Environmental Science and Health, Part B, 51, 176–181.

Feás, X., Vázquez-Tato, M.P., Estevinho, L., Seijas, J.A., Iglesias, A. (2012). Organic bee pollen: botanical origin, nutritional value, bioactive compounds, antioxidant activity and microbiological quality. Molecules, 17, 8359–8377.

Gómez-Caravaca, A.M., Gómez-Romero, M., Arráez-Román, D., Segura-Carretero, A., Fernández-Gutiérrez, A. (2006). Advances in the analysis of phenolic compounds in products derived from bees. Journal of Pharmaceutical and Biomedical Analysis, 41, 1220–1234.

Grange, J.M., Davey, R.W. (1990). Antibacterial properties of propolis (bee glue). Journal of the Royal Society of Medicine, 83, 159–160.

Gutfinger, T. (1981). Polyphenols in olive oils. Journal of American Oil Chemists' Society, 58, 966–968.

International standard. (1999). ISO 6887-1:1999, Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. International Organization for Standardization, Geneve, p. 5.

Kieliszek, M., Piwowarek, K., Kot, A.M., Błażejak, S., Chlebowska-Śmigiel, A., Wolska, I. (2018). Pollen and bee bread as new health-oriented products: A review. Trends in Food Science & Technology, 71, 170–180.

Klancnik, A., Guzej, B., Kolar, M.H., Abramovic, H., Mozina, S.S. (2009). In vitro antimicrobial and antioxidant activity of commercial rosemary extract formulations. Journal of Food Protection, 72, 1744–1752.

Kovač, J., Šimunović, K., Wu, Z., Klančnik, A., Bucar, F., Zhang, Q., Možina, S.S. (2015). Antibiotic resistance modulation and modes of action of (-)-α-pinene in *Campylobacter jejuni*. PLoS ONE 10, e0122871.

Lilek, N., Pereyra Gonzales, A., Božič, J., Kandolf Borovšak, A., Bertoncelj, J. (2015). Chemical composition and content of free tryptophan in Slovenian bee pollen. Journal of Food and Nutrition Research, 54(4), 323-333.

Martin, N.H., Trmčić, A., Hsieh, T.-H., Boor, K.J., Wiedmann, M. (2016). The evolving role of coliforms as indicators of unhygienic processing conditions in dairy foods. Frontiers in Microbiology, 7, 1549

Mauriello, G., De Prisco, A., Di Prisco, G., La Storia, A., Caprio, E. (2017). Microbial characterization of bee pollen from the *Vesuvius area* collected by using three different traps. PLoS One, 12, e0183208.

Mavri, A., Abramovič, H., Polak, T., Bertoncelj, J., Jamnik, P., Smole Možina, S., Jeršek, B. (2012). Chemical properties and antioxidant and antimicrobial activities of Slovenian propolis. Chemistry and Biodiversity, 9, 1545–1558.

Morais, M., Moreira, L., Feás, X., Estevinho, L.M. (2011). Honeybee-collected pollen from five Portuguese Natural Parks: palynological origin, phenolic content, antioxidant properties and antimicrobial activity. Food and Chemical Toxicology 49, 1096–1101.

Münstedt, K., Bogdanov, S. (2009). Bee products and their potential use in modern medicine. Journal of Apriproduct and Apimedical Science, 1(3), 57–63.

Pascoal, A., Rodrigues, S., Teixeira, A., Feás, X., Estevinho, L.M. (2014). Biological activities of commercial bee pollens: antimicrobial, antimutagenic, antioxidant and anti-inflammatory. Food Chemical Toxicology, 63, 233–239.

Sancho-Madriz, M.F. (2003). Preservation of Food, in: Caballero, B. (Ed.), Encyclopedia of Food Sciences and Nutrition (Second Edition). Academic Press, Oxford, pp. 4766–4772.

Terpinc, P., Polak, T., Makuc, D., Poklar Ulrih, N., Abramovič, H. (2012). The occurrence and characterisation of phenolic compounds in *Camelina sativa* seed, cake and oil. Food Chemistry, 131, 580–589.