Hungarian University of Agriculture and Life Sciences

CHARACTERIZATION AND COMPARISON OF MONOFLORAL BEE POLLENS BASED ON THEIR PROPERTIES RELATED TO HUMAN NUTRITION

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Rita Végh

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Doctoral School: Doctoral School of Food Sciences

Discipline: Food sciences

Head of Doctoral School Dr. Livia Simon Sarkadi

Professor, DSc

Hungarian University of Agriculture and Life Sciences

Institute of Food Science and Technology

Department of Nutrition

Supervisors: Dr. Mariann Csóka

Professor, PhD

Hungarian University of Agriculture and Life Sciences

Institute of Food Science and Technology

Department of Nutrition

Dr. László Sipos

Professor, PhD

Hungarian University of Agriculture and Life Sciences

Institute of Food Science and Technology

Department of Postharvest, Supply Chain, Commerce and Sensory Science

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Approval of the Head of School Approval of Supervisors

1. Background and objectives of the work

Due to its nutritional value and potential physiological effects, bee pollen has gained popularity among health-conscious consumers. Consequently, a recent international standard has been developed to regulate the production, quality, and testing of this product (ISO 24382:2023), and an upper limit for their pyrrolizidine alkaloid content has been established (Regulation 2023/915/EU). The importance of beekeeping products is further demonstrated by the significant increase in related international research over the past decades. These studies primarily focus on their nutritional value and potential therapeutic effects. However, numerous publications address contaminants accumulated in them, such as pesticide residues, which have received increased scientific attention due to the global bee population declines.

Thanks to the climatic and environmental conditions of the Carpathian Basin, beekeeping programs, and the traditional beekeeping expertise, currently there are more than twenty thousand beekeepers in Hungary. The indirect benefit of bees lies in their pollination activity, which contributes to the maintenance of biodiversity and agricultural production, while their direct benefit is derived from the sale of beekeeping products. In Hungary, bees visit hundreds of plant species for nectar and/or pollen, resulting in beekeeping products with diverse physical, chemical, sensory, nutritional, physiological, and food safety properties. The role of research related to bees and beekeeping products has been increasingly valued in recent years, as the sector faces significant challenges, including factors threatening bee health and loss of economic competitiveness due to the rising production costs and import of cheap, low-quality honeys.

In recent years, many studies have been conducted on the physical-chemical properties and authenticity of Hungarian honeys; however, limited scientific information is available in Hungarian language on other beekeeping products, which has led to misconceptions about their nutritional properties. During my doctoral work, I comprehensively evaluated the nutritionally relevant characteristics of bee pollens from various botanical sources, scientifically grounding their nutritional-physiological properties, food safety risks, sensory characteristics, and their applicability as functional food ingredients. My personal motivation was to help dispel misconceptions related to bee pollen consumption and to support beekeepers' work and consumers' informed decision-making by providing credible information.

The main goal of my work is a complex evaluation of pollen pellets from nutritional-physiological, food safety, and sensory perspectives. Another objective is to assess their effectiveness as functional food ingredients through the enrichment of biscuits, with special attention to the impact of botanical origin.

The research objectives are presented in sub-goals below:

- A Hungarian-language summary and critical analysis of international literature related to the role of bee pollen in human nutrition, in order to help dispel misconceptions surrounding the product and to promote wider access to scientifically based knowledge.
- Identification, estimation, and evaluation of the food safety risks associated with bee pollen consumption.
- Creation of monofloral bee pollen samples from wildflowers and cultivated plants characteristic of the domestic flora, and identification of their botanical origin.
- Determination of the color characteristics,
- macronutrient and amino acid composition,
- total phenolic content and flavonoid content,
- as well as antioxidant capacity of the monofloral bee pollen samples using *in vitro* methods.
- Validation of a multi-residue liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for bee pollen matrix: determining limit of detection, limit of quantification, linearity, precision, recovery, and matrix effect. Analysis of pesticide residue content in samples using the validated method. Conducting a human food safety risk assessment.
- Optimization of headspace solid-phase microextraction (HS-SPME) parameters for determining volatile components of bee pollens. Qualitative and semi-quantitative analysis of the volatile fraction of samples with gas chromatography-mass spectrometry-olfactometry (GC-MS-O). Identification of aroma-active compounds and potential botanical markers.
- Enrichment of biscuits with bee pollens derived from monoculture plants frequently visited by honey bees in various concentrations. Examination and comparison of the biscuits' nutritional value, color parameters, sensory profile, and consumer acceptance. Determination of further directions for product development using preference mapping and penalty analysis.

2. Materials and methods

2.1. Literature review and risk assessment

A literature review related to the food safety risks of pollen pellets was conducted between 2020 and 2021. The publications used were sourced from electronic databases of English-language scientific journals. The keywords used in the research were: "bee pollen," "apiculture product," "food safety," "pesticide residue," "heavy metal," "toxic element," "mycotoxin," "pyrrolizidine alkaloid," "allergen," "case study," and "risk assessment." Only studies relevant to the topic and published after 2000 (except case studies) were considered. The data were presented in tables showing average and, in some cases, maximum concentration values. Exposure estimation was carried out according to **Equation 1**, considering published average concentration values (for chronic exposure estimation) or concentrations above 2 mg/kg (for acute exposure estimation). The estimation was based on a daily intake of 25 g and an average body weight of 70 kg.

Equation 1: $Daily\ exposure\ (mg/ttkg) = \frac{concentration\ (mg/kg)}{1000} \times 25/70$

For non-genotoxic carcinogenic components, the risk quotient was calculated by dividing the exposure by the currently established reference value for the component (**equation 2**). For pesticides, acceptable daily intake (ADI) and the acute reference dose (ARfD) were used as reference values. The reference values were sourced from the EU pesticide database. For non-genotoxic carcinogenic elements (Cd, Hg) and mycotoxins (ochratoxin-A, fumonisins, zearalenone, deoxynivalenol, T2-toxin), health-based guidance values, such as provisional tolerable daily, weekly, or monthly intake values (PTDI, PTWI, PTMI) have been established, so these reference values were used in the calculations. In the chronic risk assessment, a risk quotient above 0.1 was considered significant, as pollen pellet consumption generally constitutes a small portion of daily energy intake. For the acute risk assessment, a value above 0.5 was considered significant.

Equation 2: $Risk\ quotient = \frac{Exposure}{Reference\ value}$

For genotoxic carcinogenic components, such as arsenic, lead, aflatoxin B1, and pyrrolizidine alkaloids, health-based guidance values cannot be established. In these cases, the Margin of Exposure (MoE) approach is recommended (EFSA, 2019). The basis of the MoE is a

reference point called the BMDL (benchmark dose lower confidence limit), which indicates a dose that results in a predetermined response change (e.g., a 10% increase in the occurrence of a certain tumor type) relative to the background response. The MoE is the ratio of the BMDL value to the estimated human exposure. The MoE is a unitless number, where a lower calculated MoE value corresponds to greater public health concern due to exposure to the substance. Based on the established reference values and recommended minimum MoE values, a "theoretical maximum exposure" was determined, against which the estimated exposure values were compared. Detailed data related to the risk assessment method can be found in our literature review (Végh *et al*., 2021).

2.2. Experimental fundamental research: Physicochemical characterization and comparison of Monofloral bee pollens

2.2.1. Sample collection

In the research, bee pollen samples were obtained from domestic beekeepers and a specialty store selling beekeeping products. These formed the basis of the monofloral samples (**Figure 1**), which were prepared by manually sorting the pellets. Sorting was primarily based on their color, but pellet shape and size also provided useful information in some cases.

Figure 1. Monofloral bee pollen samples used in the experiments

2.2.2. Experimental methods

- **Botanical origin**: microscopic pollen analysis
- **Color parameters**: CIELAB color analysis on ground samples
- **Crude protein content**: classical Kjeldahl-method (nitrogen-protein conversion factor: 5,6)
- **Crude fat content**: classical Soxhlet-method using petroleum ether
- **Ash content**: gravimetric determination after ashing at 560 °C

- **Moisture content**: gravimetric determination after vacuum drying (0,05 MPa, 75 °C)
- **Total carbohydrate content**: estimation by calculation
- **Amino acid composition**: ion exchange chromatography
- **Total phenolic content**: spectrophotometric method
- **Total flavonoid content**: spectrophotometric method
- **Antioxidant capacity**: spectrophotometric methods, namely
  Ferric Reducing Antioxidant Power (FRAP) assay
  Cupric Ion Reducing Antioxidant Capacity (CUPRAC) assay
  2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay
  2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

- **Pesticide residue content**: Determined using a multi-residue HPLC-MS/MS method developed at the Department of Food Chemistry and Analysis, MATE. The method was previously validated for the bee pollen matrix according to SANTE criteria.
- **Volatile and aroma-active components**: Determined using a method developed at the Department of Nutrition Science, MATE, applying GC-MS coupled with an olfactometer. Volatile components were extracted from the sample's headspace by solid-phase microextraction (HS-SPME), with parameters optimized for the bee pollen matrix.

2.3. Practical research: Determination of directions for the development of biscuits enriched with bee pollens

2.3.1. Preparation of biscuits

Biscuits (**Figure 2**) were prepared according to the recipe approved by the American Association of Cereal Chemists (AACC, 1980), by substituting the flour with ground bee pollen derived from rapeseed (*Brassica napus*), sunflower (*Helianthus annuus*), and phacelia (*Phacelia tanacetifolia*) at 2%, 5%, and 10%.

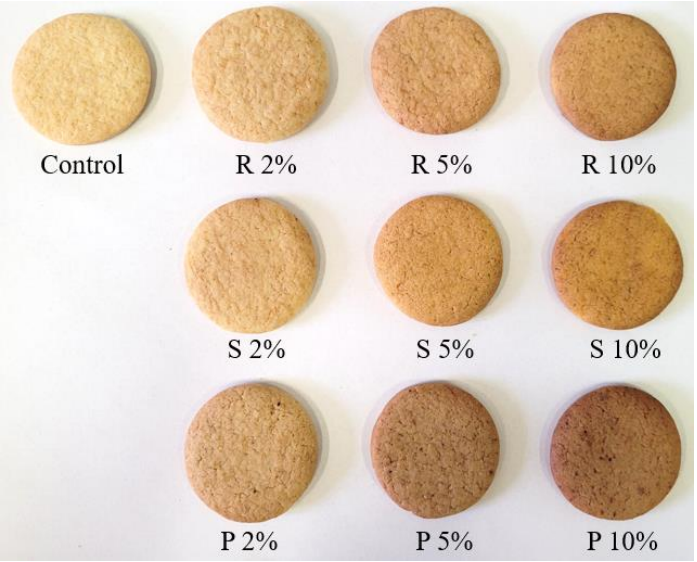


Figure 2. Control sample and biscuits enriched with rapeseed (R), sunflower (S), and phacelia (P) bee pollens

1.3.2. Experimental methods

- **Color parameters**: CIELAB color measurement on the surface of biscuits
- **Crude protein content**: classical Kjeldahl-method (nitrogen-protein conversion factor: 6,25)
- **Crude fat content**: classical Soxhlet-method using petroleum ether
- **Ash content**: gravimetric determination after ashing at 525 °C
- **Moisture content**: gravimetric determination after drying at 105 °C
- **Total carbohydrate content**: estimation by calculation
- **Total phenolic content**: spectrophotometric method
- **Antioxidant capacity**: spectrophotometric methods, namely:
  Ferric Reducing Antioxidant Power (FRAP) assay
  2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay
  2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay
- **Sensory profile**: quantitative descriptive analysis (QDA); number of assessors: 12
- **Consumer preference**: 9-point monotonically increasing preference scale, 5-point JAR (Just About Right) scale; number of consumers: 100
- **Relationship between sensory profile and consumer liking:** preference mapping
- **Determination of directions to increase consumer acceptance:** penalty analysis

1.4. Statistical methods

- For small sample sizes (<10): Kruskal–Wallis test with Dunn's pairwise comparisons and Bonferroni correction (α=0.05)*
- For large sample sizes ($\geq$10), if normality and homogeneity of variance assumptions are met: ANOVA with Tukey-HSD post hoc test (α=0.05)
- Spearman correlation analysis (α=0.05) for analyzing correlations between color and antioxidant parameters
- Hierarchical cluster analysis (HCA) to create consumer groups based on their preferences
- Principal component analysis (PCA) for dimension reduction of preference and product data

*Note: This test is based on the ranks of the obtained values, so the magnitude of differences between the data is not reflected, which can significantly reduce the sensitivity of the test. The results should be interpreted with consideration of this limitation.

3. Results and discussion

3.1. Results of literature review and risk assessment

During the literature review, primary scientific findings related to the risks of consuming bee pollens have been analyzed and synthesized, followed by a food safety risk assessment. The assessment revealed significant risks associated with arsenic, cadmium, lead, aflatoxin, and pyrrolizidine alkaloid content in bee pollens. The latter conclusion is supported by the recent establishment of maximum limits for pyrrolizidine alkaloids in pollens (Regulation 2023/915/EU). Case studies of allergic reactions induced by bee pollen consumption indicate that the product can cause symptoms suggestive of anaphylaxis (facial edema, swelling of the mouth, tongue, and throat, difficulty of swallowing and breathing, sore throat, itching, urticaria, malaise) in sensitive individuals. These symptoms were mostly triggered by products containing dandelion or other *Asteraceae* pollens, which exhibit cross-reactivity with the pollen of mugwort (*Artemisia vulgaris*), a frequent cause of respiratory allergies.

3.2. Botanical, color, and nutritional characteristics of monofloral bee pollen samples

The experimental research focused on 14 monofloral bee pollen samples, each containing over 80% predominant pollen (ISO 24382:2023). These included both cultivated plants (rapeseed, sunflower, phacelia, sweet cherry) and wild flora species (wild blackberry, dandelion, red poppy, rock-rose, traveller's joy, dropwort, honey locust, musk thistle). Three different apiaries provided rapeseed pollens, allowing the investigation of the effects of geographical origin.

Color analysis indicated a dominance of red and yellow hues over green and blue, owing to their carotenoid and flavonoid content. The crude protein (13.01–23.96%), ash (1.05–3.24%), and fat contents (1.40–10.52%) met the recently published standard criteria. Rapeseed and phacelia pollens had the highest crude protein content; sweet cherry and musk thistle pollens had elevated ash content, and dandelion pollen had the highest crude fat content. The essential amino profiles of traveller's joy and red poppy pollens met human nutritional requirements the most, while the rock-rose pollen had the least balanced essential amino acid composition (**Figure 3**).

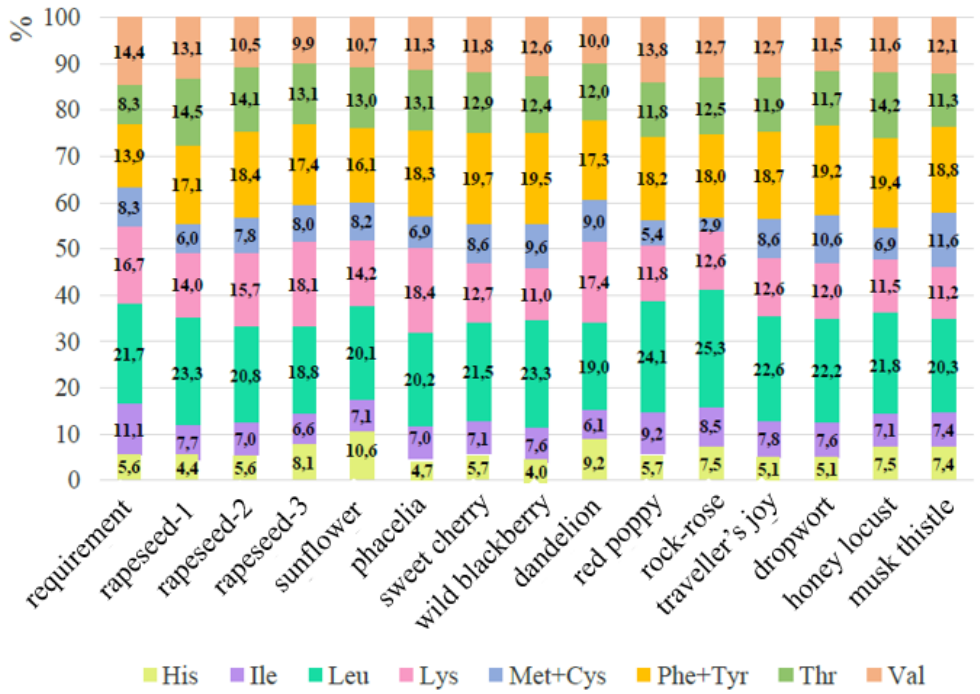


Figure 3. Essential amino acid profiles of bee pollen samples compared to the reference protein

Using a pollen mixture, I selected the solvent yielding the highest total phenolic content from distilled water and organic solvents (methanol, ethanol, acetone) at various concentrations. The highest extraction was achieved by using 60% ethanol. This solvent was used for antioxidant property assays. Total phenolic content ranged from 9.82 to 25.31 mg gallic acid equivalents (GAE)/g sample, and total flavonoid content varied between 0.83 and 10.51 mg quercetin equivalents (QE)/g sample. The flavonoid-to-phenolic ratio showed high variability, from 9% (honey locust pollen) to 44% (traveller's joy pollen). Based on *in vitro* antioxidant capacity assays (FRAP, CUPRAC, ABTS, DPPH), relative antioxidant capacity indices were calculated and ranked (**Figure 4**). Based on our results, pollens of rapeseed and traveller's joy had high relative values, in contrast to the members of the *Asteraceae* family, namely sunflower, musk thistle, and dandelion. The antioxidant capacity index correlated strongly with the total phenolic content and antioxidant capacity assay results, confirming its suitability for characterizing the antioxidant potential in pollen samples. Contrary to previous findings, no positive correlation was found between color parameters and antioxidant capacity.

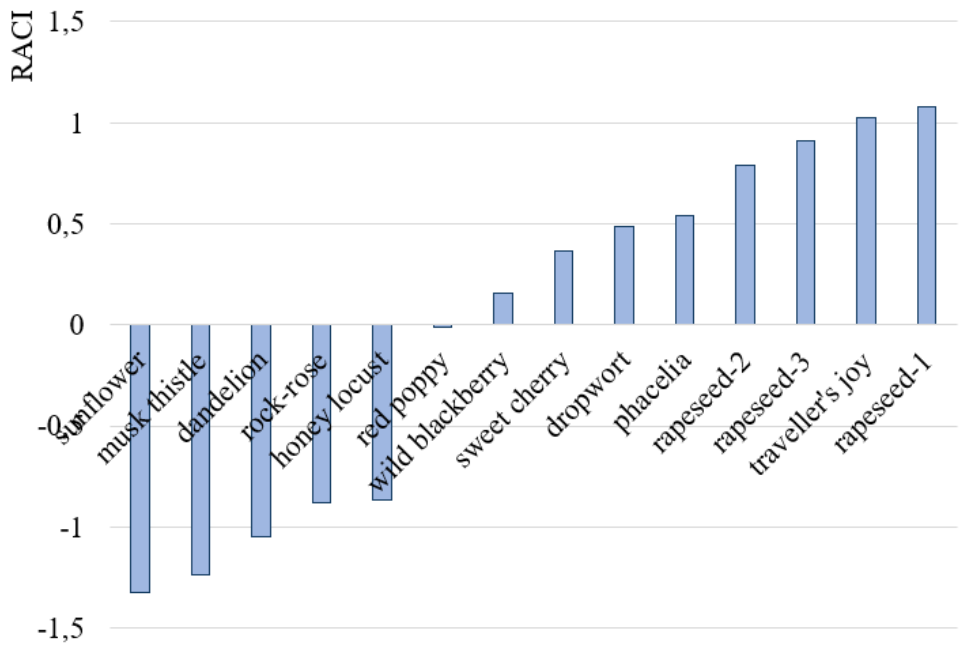


Figure 4. Relative antioxidant capacity indices of bee pollens

3.3. Pesticide residue content of bee pollens

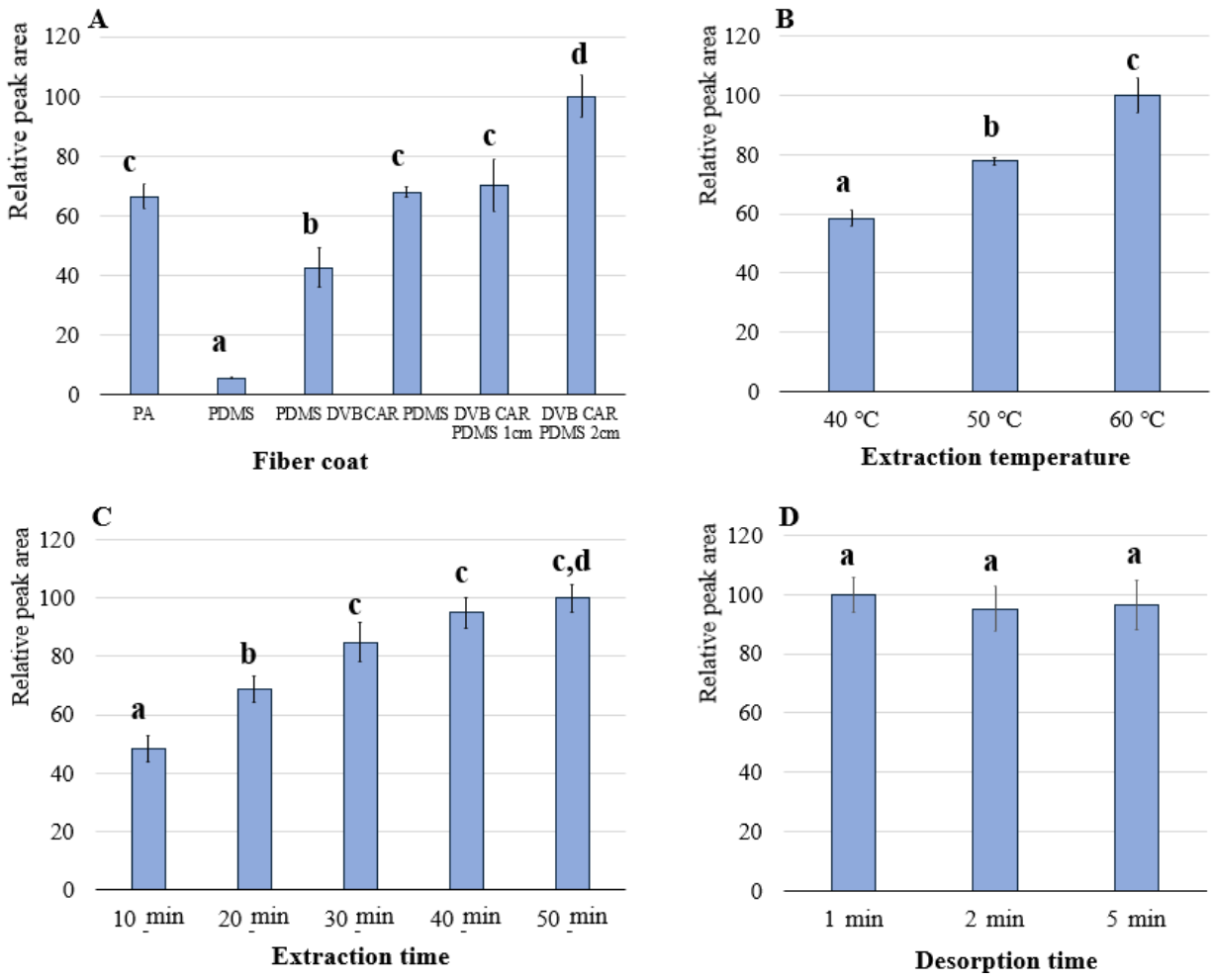
A multi-residue analytical method was validated for bee pollen matrix. Based on the achieved performance characteristics, 247 analytes met the SANTE criteria. Limits of detection ranged from 1.25 to 15.63 µg/kg. Limits of quantification were 6.5 µg/kg for most compounds, and never exceeded 32.5 µg/kg. Calibration curves showed high correlation coefficients ($r^2 > 0.990$) except for six compounds. The lowest correlation coefficient ($r^2 = 0.965$) was observed in the case of oxadixyl. For 85% of the analytes, matrix effects between 80% and 120% (within the predefined criteria) were observed; however, in some cases, this parameter was extremely high (>200%) or low (<30%). These results suggest that the matrix effect is strong, necessitating the use of matrix-matched calibration. The obtained average recovery values met the 70-120% criterion in the majority of cases. However, lower recoveries were recorded for 15 analytes, which require continuous monitoring and correction during method application (on-going validation). The intra-day repeatability proved adequate in all cases, as relative standard deviations were below 20%.

The pesticide residue content of 14 monofloral bee pollens was analyzed using the validated method. Nearly half of the samples contained at least one active substance at a concentration above the LOQ. Based on my results, active substances are likely to be detected in products derived from cultivated plants, particularly rapeseed, in contrast to pollen from wildflowers and trees. In total, 12 active substances were detected in the samples, mainly insecticides and fungicides, of which 7 substances (thiacloprid, chlorpyrifos, indoxacarb, cyproconazole, dimoxystrobin, propamocarb,

linuron) are currently not approved for use in the European Union. Results of risk assessment revealed that the acute and chronic food safety risks associated with the pesticide residues detected in the samples are negligible.

3.4. Volatile fraction and aroma-active components of bee pollens

Before analyzing the volatile compounds of bee pollens, the extraction parameters were optimized. The most efficient extraction was achieved using a 50/30 µm DVB/CAR/PDMS fiber coating (2 cm in length), an extraction temperature of 60 °C, an extraction time of 30 minutes, and a desorption time of 1 minute. These results are illustrated in **Figure 5.**



5. ábra: Optimization of HS-SPME parameters for the extraction of volatiles from bee pollen based on total peak areas

Different letters indicate significant differences between groups ($p < 0,05$)

The number of detected volatiles was between 75 and 101 in the samples. In terms of compound numbers, terpenes/terpenoids and esters were the most prevalent classes, while in terms of relative area, acids and esters dominated in most of the samples. An exception was phacelia pollen, which contained remarkably high amounts of aldehydes and ketones. During the work, unique volatiles have been identified, which were present exclusively in one sample. Besides, non-species-specific markers have also been identified, which showed exceptionally high proportions only in certain samples. For example, hexanal and 3,5-octadien-2-one were detected in high relative abundance exclusively in phacelia pollen, while *α*-pinene was characteristic only of sunflower pollen. Lilac aldehydes and lilac alcohols were detected exclusively in cherry pollen, while degradation products of *cis*-geraniol were characteristic of poppy pollen. These compounds may serve as potential botanical markers for the chemical traceability of the products.

In total, 296 different volatiles were identified in the 14 samples, more than half of which were also detectable by olfactometry in at least one product. **Figure 6** illustrates the distribution of perceived odor notes. This figure serves to provide a general overview of the odor of bee pollens, and to illustrate the complexity of the perceived odor notes. However, it is important to note that substantial differences were observed among the samples in terms of the composition and intensity of odor-active compounds.

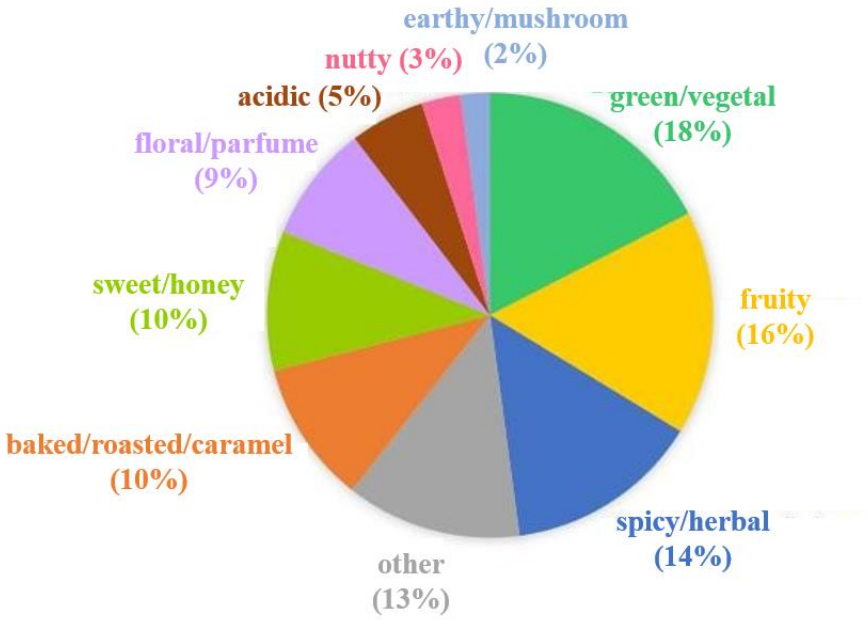


Figure 6. Distribution of perceived odor notes in 14 bee pollens

3.5. Physical, chemical, and sensory properties of biscuits enriched with monofloral bee pollens

In the product development phase of the work, the physicochemical properties, sensory profiles, and consumer acceptance of biscuits enriched with bee pollens from rapeseed, sunflower, and phacelia were analyzed. The results of the research revealed that phacelia pollen improved the nutritional properties of the biscuits the most. However, in terms of consumer preference, sunflower pollen proved to be the most favorable for enrichment, especially at the 10% concentration level. The main reason for this is presumably that the biscuits enriched with rapeseed pollen had a cabbage-like odor and taste, while the phacelia pollen-enriched biscuits exhibited a slight aroma reminiscent of cut hay, according to the expert sensory panel. A preference map, generated by combining consumer preference data with intensity ratings from expert profile analysis, indicated that consumers tended to prefer the control samples and those with lower pollen content (**Figure 7**). Since a 10% pollen addition was necessary to achieve a significant improvement in the nutritional value of the biscuits, a penalty analysis was performed to determine how the consumer acceptance of these biscuits could be improved. Based on our findings, this would require the development of a formulation that reduces the overall taste, overall odor, color intensity, and hardness of the biscuits, while increasing their sweet taste and sweet odor intensity.

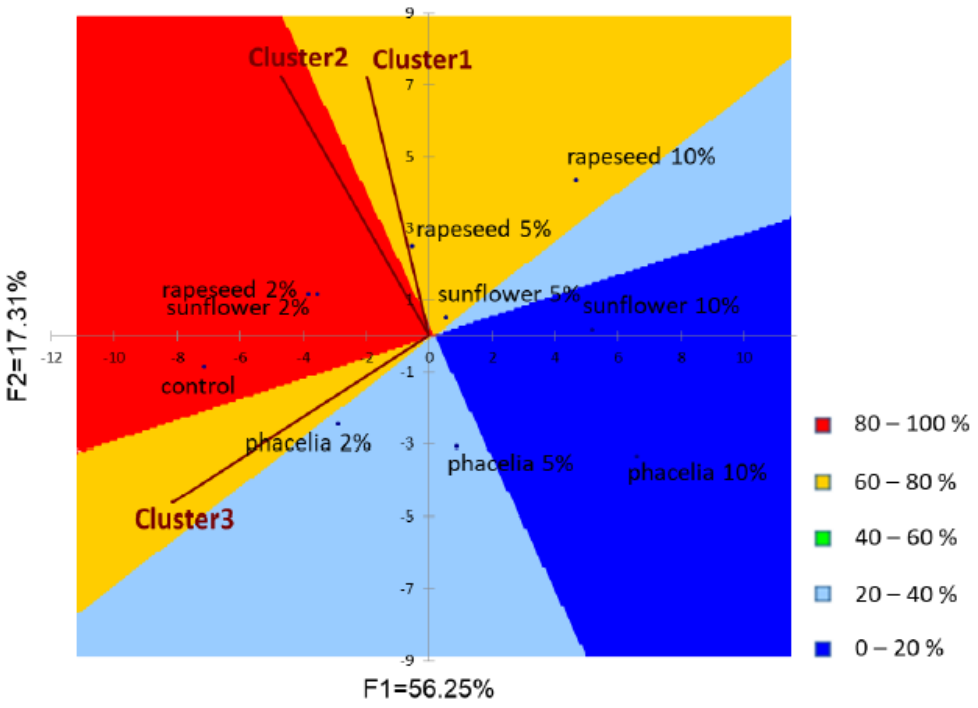


Figure 7. Preference map of biscuits enriched with bee pollens of different botanical origins

4. Conclusions and recommendations

In the first phase of my research, a literature review was conducted on the food safety risks associated with pollen consumption, including the presence of pesticide residues, toxic elements, mycotoxins, pyrrolizidine alkaloids, and allergens. This work was complemented by risk assessments, which indicated that the risks arising from arsenic, cadmium, lead, pyrrolizidine alkaloid, and aflatoxin contamination in the products are significant. Case studies revealed that allergic symptoms are most frequently caused by pollen from dandelion or other taxa belonging to the *Asteraceae* family, which show cross-reactivity with the pollen of mugwort (*Artemisia vulgaris*), a common trigger of respiratory allergies. In the light of these findings, it is necessary to promote the bee pollen standard and to educate beekeepers about potential food safety hazards, since proper beekeeping practices can minimize these risks. To protect consumers, regular monitoring and official control of the products are recommended, and the establishment of maximum limits may also be justified. Furthermore, it is advisable to introduce mandatory warning labels on product packaging related to the potential allergenicity of bee pollen.

My experimental results confirm that bee pollens of different botanical origins show significant variability in color and chemical parameters. The crude protein, ash, and fat contents of the samples were in line with the recently published standard criteria. However, generally lower crude protein contents were found compared to the literature data. This is primarily attributable to the fact that protein contents are often overestimated in studies due to the use of the general nitrogen-to-protein conversion factor (6.25) instead of the specific factor (5.6) prescribed in the bee pollen standard. Samples deriving from rapeseed and phacelia were rich in proteins and phenolic compounds, had a relatively balanced amino acid composition, and exhibited high antioxidant capacity in *in vitro* tests. Products from the *Asteraceae* family showed less favorable nutritional and physiological properties. These results align with several other research findings, suggesting their general validity. The relative antioxidant capacity index strongly correlated with the amount of phenolic compounds and the results of antioxidant capacity assays, confirming that this approach is suitable for characterizing the antioxidant potential of pollen samples. Contrary to a previous study, no positive correlation was found between color and antioxidant parameters.

During the research, a multi-residue method was validated for bee pollen matrix. The performance characteristics of 247 analytes met the SANTE validation criteria. In agreement with literature data, strong matrix effects were observed for several analytes; therefore, matrix-matched calibration is recommended for pesticide analysis of bee pollen samples. Using the validated method, the pesticide residue content of bee pollen samples included in this research was analyzed. The results indicate that residues of active substances are more likely to be detected in products

derived from cultivated plants – particularly rapeseed – than in those originating from wildflowers or trees. Many of the active substances identified in the samples, such as thiacloprid, chlorpyrifos, and hexaconazole, are currently not authorized in the European Union due to potential health and/or environmental aspects. Nevertheless, the acute and chronic food safety risks associated with the detected pesticide residues were found to be low.

With respect to volatile compound analysis, extraction was most effective under the following conditions: DVB/CAR/PDMS fiber coating (50/30 μm, 2 cm length), extraction temperature of 60 °C, extraction time of 30 minutes, and desorption time of 1 minute. Using these optimized parameters, between 75 and 101 volatile compounds were identified in the bee pollen samples. Each sample contained at least one unique volatile component, which may serve as a potential botanical marker. However, in the case of rapeseed pollen, no source-specific characteristics were observed, suggesting that volatile profile analysis is likely unsuitable for the identification of rapeseed origin. More than half of the identified volatile compounds were olfactometrically detectable in at least one sample. The odor-active compounds were predominantly characterized by “green/vegetal,” “fruity,” “spicy/herbal,” “baked/roasted/caramel,” “sweet/honey,” or “floral/perfume” notes. These odors varied considerably in both intensity and character between samples of different botanical and geographical origins, indicating that the aroma compounds contribute in diverse and complex ways to the unique aroma profiles of bee pollens. These findings underscore the significant influence of botanical origin on the sensory attributes and, consequently, on the consumer acceptance of pollen-enriched food products.

The product development phase applied these findings in practice by examining the chemical, physical, and sensory properties of biscuits enriched with rapeseed, sunflower, and phacelia bee pollens. The results revealed that phacelia pollen was the most effective in improving the nutritional quality of the biscuits, whereas sunflower pollen was the most effective in enhancing consumer acceptance. Preference mapping demonstrated that consumers most favored biscuits with low pollen content and the control sample, while those with high pollen content were least preferred. These findings were consistent with the results of optimum scaling, which indicated that cookies containing 2% sunflower pollen received the highest "just-about-right" ratings. Although cookies with 10% pollen content exhibited the most favorable nutritional and physiological properties, they achieved the lowest levels of consumer acceptance. Penalty analysis revealed that to enhance consumer preference for high-pollen cookies, product formulations should aim to reduce overall flavor intensity, aroma, color intensity, and hardness, while increasing the intensity of sweet taste and sweet odor.

As all scientific research, the present study raises further questions that merit future investigation. The above findings support that bee pollens are rich sources of diverse nutrients and bioactive compounds. However, comprehensive *in vivo* and *in vitro* studies are required to assess the digestibility and bioavailability of nutrients from pollens of different botanical origins. Given that pollen grains differ in their cell wall structures depending on plant species, their utilization efficiency likely varies as well, although currently, little information is available on this subject. Furthermore, additional research is needed to evaluate the applicability of alternative technologies, such as high hydrostatic pressure (HHP) or pulsed electric field (PEF), for improving the digestibility of pollen. Further investigations on the food safety parameters of pollen pellets would also be valuable, particularly regarding toxic elements, microplastics, pyrrolizidine alkaloids, and mycotoxins. The identification of allergenic and potentially cross-reactive proteins in pollens from the *Asteraceae* family also represents an important research direction. Results of the present dissertation clearly show that bee pollens from different botanical sources possess distinct volatile compound profiles, warranting further research on identifying botanical markers and developing validated methods for origin determination. Finally, the outcomes of penalty analysis also suggest promising research directions to enhance consumer acceptance of biscuits with high pollen content.

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5. New scientific results

1. By analyzing literature data, secondary research was conducted on the food safety risks associated with bee pollen consumption. Risk assessment confirmed that the chronic risk due to lead, cadmium, arsenic, pyrrolizidine alkaloid, and aflatoxin contamination in these products is significant, whereas the pesticide residues often detected in them pose low human risk. Research on allergic case studies related to bee pollen consumption revealed that generally pollen from dandelion (*Taraxacum officinale*) or other species belonging to the *Asteraceae* family trigger these reactions.

2. For the first time, monofloral bee pollens from of domestic cultivated plants and wildflowers, many with previously unexplored chemical properties, were profiled based on their nutritional and physiological characteristics. Isoleucine or lysine was found to be the limiting amino acid in 93% of the products for adult consumers. The relative antioxidant capacity approach was applied to bee pollens for the first time, revealing that samples from rapeseed (*Brassica napus*) and traveller's joy (*Clematis vitalba*) exhibit high antioxidant potential, in contrast to pollens from sunflower (*Helianthus annuus*), musk thistle (*Carduus nutans*), and dandelion (*Taraxacum officinale*),

3. A new multi-residue analytical method was validated for the determination of pesticide residue content in bee pollens. The method's performance characteristics, including limits of detection (LOD), limits of quantification (LOQ), linearity, precision, recovery, and matrix effects, met predefined criteria for 247 active substances. This method was applied for the first time to domestic bee pollens, with at least one pesticide detected in every sample originating from cultivated plants. A total of 12 active substances were identified, seven of which have recently been banned within the European Union due to human health and/or ecotoxicological concerns. Risk assessment indicated that acute and chronic food safety risks posed by pesticide residues in the samples are negligible.

4. A headspace solid-phase microextraction (HS-SPME) method was developed and optimized for the gas chromatographic analysis of volatile compounds in bee pollens. Optimal extraction parameters were determined as follows: fiber coating of DVB/CAR/PDMS (50/30 μm, 2 cm), extraction temperature of 60 °C, extraction time of 30 minutes, and desorption time of 1 minute. Using this sampling technique, the volatile compound composition of domestic pollen samples was studied for the first time by gas chromatography–mass spectrometry coupled

with olfactometry (GC-MS-O), combining instrumental identification with sensory odor characterization. The perceived odor notes generally grouped into “green/vegetal,” “fruity,” “spicy/herbal,” “baked/roasted/caramel,” “sweet/honey,” and “floral/perfume” categories. It was also demonstrated that the volatile and aroma-active components of samples from different source plants show significant differences in both quantity and quality, and that some compounds can be used as species-specific markers to help botanical identification.

5. It was demonstrated for the first time that biscuits enriched with bee pollens from rapeseed (*Brassica napus*), sunflower (*Helianthus annuus*), and phacelia (*Phacelia tanacetifolia*) exhibit distinct physical, chemical, and sensory characteristics. Among these, phacelia pollen was identified as the most effective ingredient for enhancing nutritional properties. Biscuits enriched with phacelia pollen displayed a hay-like flavor and aroma, with intensity increasing proportionally to pollen concentration (2%, 5%, and 10%), which negatively impacted consumer acceptance. In contrast, sunflower pollen addition resulted in the most favorable consumer liking across all tested concentrations. By combining consumer preference data with expert sensory profile analysis, a preference map was created, and penalty analysis was conducted for determining further directions of product development of the 10% pollen-enriched biscuits.

6. List of publications in the field of studies

Book chapter

Rita Végh, Mariann Csóka (2024). Amino acids, peptides and proteins of pollen. Chapter in: Nesrin Ecem Bayram, Aleksandar Z. Kostic & Yusuf Can Gercek (Editors), *Pollen – Chemistry and Biotechnology*. Springer Nature Switzerland AG. ISBN: 978-3-031-47563-4, https://doi.org/DOI: 10.1007/978-3-031-47563-4

Articles

Mariann Csóka, **Rita Végh,** László Sipos (2025). Volatile Profile of Bee Pollens: Optimization of Sampling Conditions for Aroma Analysis, Identification of Potential Floral Markers, and Establishment of the Flavor Wheel. *Food Science & Nutrition*, 13, e4707 (Q1, IF: 4,667)

Rita Végh, Mariann Csóka, Csilla Sörös, László Sipos (2024). Underexplored food safety hazards of beekeeping products: Key knowledge gaps and suggestions for future research. *Comprehensive Reviews in Food Science and Food Safety*, 23, e23404. (D1, IF: 15,679)

Rita Végh, Gizella Sipiczki, Mariann Csóka (2023). Investigating the antioxidant and colour properties of bee pollens of various plant sources. *Chemistry & Biodiversity*, 20, e202300126. (Q2, IF: 2,468)

Rita Végh, Mariann Csóka, Éva Stefanovits-Bányai, Réka Juhász, László Sipos (2023). Biscuits enriched with monofloral bee pollens: nutritional properties, techno-functional parameters, sensory profile, and consumer preference. *Foods,* 12, 18. (Q1, IF: 5,077)

Végh Rita, Puter Dorina, Vaskó Áron, Csóka Mariann, Mednyánszky Zsuzsanna (2022). Mézek és virágporok beltartalmi összetételének és színjellemzőinek a vizsgálata. *Élelmiszervizsgálati Közlemények*, 68, 3779-3792. (Q4, IF: 0,220)

Rita Végh, Csilla Sörös, Nándor Majercsik, László Sipos (2022). Determination of Pesticides in Bee Pollen: Validation of a Multiresidue High-Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry Method and Testing Pollen Samples of Selected Botanical Origin. *Journal of Agricultural and Food Chemistry*, 70, 1507-1515. (D1, IF: 6,173)

Rita Végh, Mariann Csóka, Csilla Sörös, László Sipos (2021). Food safety hazards of bee pollen – a review. *Trends in Food Science and Technology*, 114, 490-509. (D1, IF: 15,643)

László Sipos, **Rita Végh**, Zsanett Bodor, John-Lewis Zinia Zaukuu, Géza Hitka, György Bázár, Zoltán Kovács (2020). Classification of bee pollen and prediction of sensory and colorimetric Attributes—A sensometric fusion approach by e□nose, e□tongue and NIR. *Sensors*, 20, 6768. (Q2, IF: 4,621)

Conference abstracts

Végh Rita, Sipiczki Gizella, Csóka Mariann. Különböző módon szárított, monoflorális virágporcsomók összpolifenol- és flavonoidtartalmának a vizsgálata. Magyar Agrártudományi Doktoranduszok Szimpóziuma. Debrecen, 2023. február 24-25.

Rita Végh, Mariann Csóka, Éva Stefanovits-Bányai, László Sipos. Effect of enrichment with monofloral pollens on the total phenolic and flavonoid content of baked goods. 4th International Conference on Food Science and Technology. 10-12 June, 2022, Budapest.

Végh Rita, Stefanovitsné Bányai Éva, Csóka Mariann, Sipos László. Különböző repce pollen extraktumok antioxidáns tulajdonságainak a vizsgálata. Magyar Haemorheologiai Társaság XXVIII., Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság, Magyar Szabadgyök-Kutató Társaság VII. Közös Kongresszusa. Pécs, 2022. április 22-23.

Rita Végh, Dornia Puter, Áron Vaskó, Mariann Csóka, Zsuzsanna Mednyánszky. Chemical composition of Hungarian and foreign apicultural products. 4th International Conference on Biosystems and Food Engineering. 4th June, 2021, Budapest.