

Rita Végh¹, Mariann Csóka¹, Éva Stefanovits-Bányai², László Sipos³

¹ Department of Nutrition, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary

² Department of Food and Analytical Chemistry, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary

³ Department of Postharvest and Sensory Science, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary

Introduction

Research background

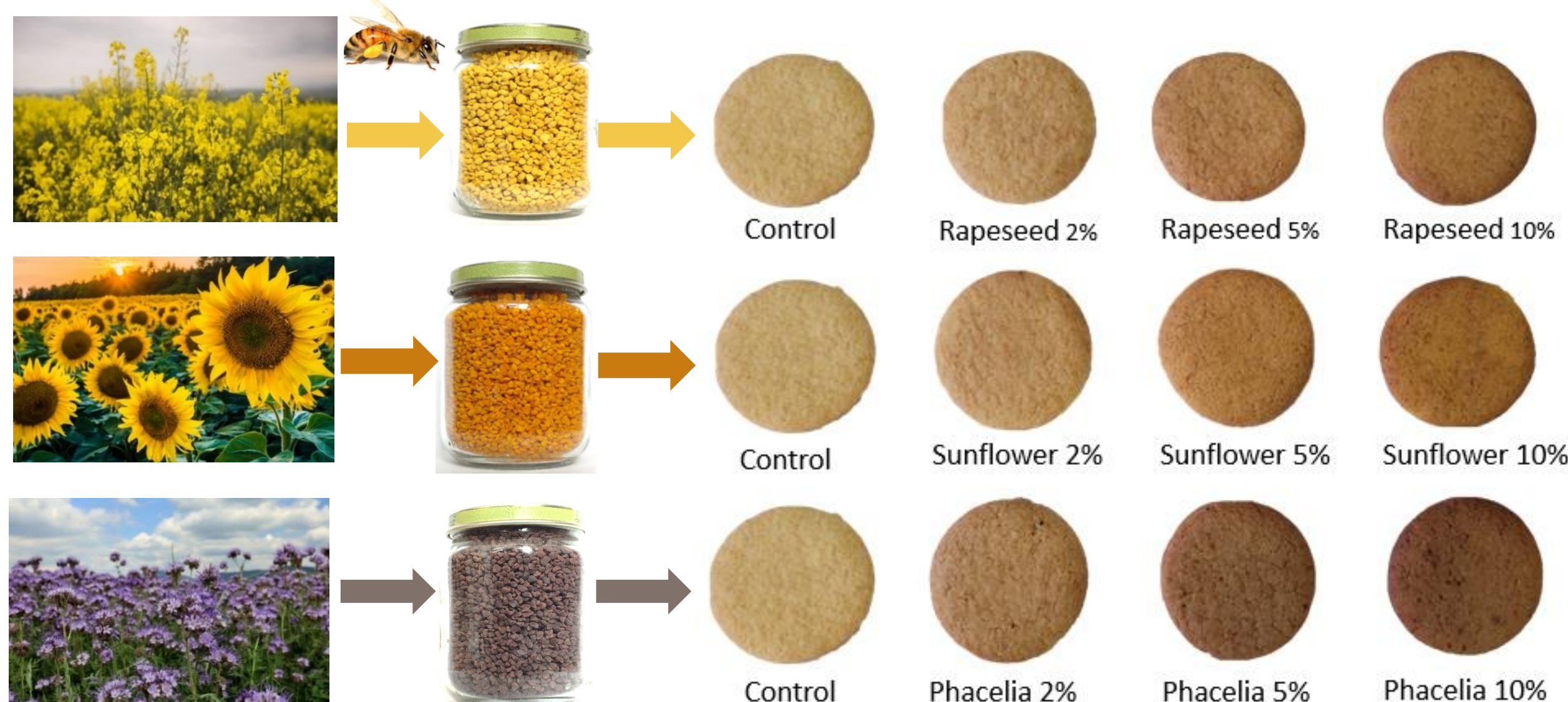
Owing to their potential health benefits, dietary antioxidants have become the focus of consumers' attention. Functional foods enriched with ingredients rich in these phytonutrients are increasingly popular. Due to its nutritional properties and therapeutic potential, bee pollen has been used as a functional food ingredient in many studies. Although the physical, chemical and sensory properties of pollens are significantly influenced by the plant source, the botanical origin of pollens used for fortification has been discussed in only a few studies, suggesting that researchers generally use polyfloral pollen pellets for this purpose.

Objectives

The aim of our work is to compare the total phenolic and flavonoid content of biscuits enriched with monofloral bee pollens originated from rapeseed (*Brassica napus* L.), sunflower (*Helianthus annuus* L.) and phacelia (*Phacelia tanacetifolia* Benth.). These plant sources were selected because they are grown monoculture, they are very attractive pollen sources for bees, and they have economic significance in many countries including Hungary.

Materials

Biscuits were prepared according to the AACC-Approved method 10-50D (AACC, 1980) by using the following ingredients: wheat flour, ground sugar, margarine, salt, distilled water, glucose syrup, baking soda. The proportions of pollens supplemented the flour were 2, 5 and 10%. All ingredients were mixed to a homogenous mass. The dough was then sheeted to 6 mm thickness. Biscuits with 50 mm diameter were formed, which were baked at 205 °C for 10 minutes. The samples were then cooled for 30 minutes, packed in sealable plastic bags and stored at room temperature.



Methods

Preparation of extracts

For the determination of the total phenolic content (TPC) and total flavonoid content (TFC) of bee pollens and biscuits, extracts were prepared as the followings: 0.20 g of bee pollen or 1.00 g of biscuit were weighed and dissolved in 10 ml of methanol:distilled water:formic acid (60:39:1). Followingly, extracts were sonicated for 1 hour, then centrifuged at 12,000 rpm for 10 minutes. The supernatant was transferred to Eppendorf tubes and stored at -20 °C. In order to evaluate the potential effects of the products on the human organism, 100 % aqueous extracts were also prepared for TPC determination.

Determination of the total phenolic content (TPC)

The total phenolic content of the pollens and biscuits were determined by the method proposed by Singleton & Rossi (1965). Folin-Ciocalteu reagent was used for preparing the solutions, the absorbances of which were determined spectrophotometrically at 760 nm. Results were expressed as mg GAE (gallic acid equivalent)/100 g sample.

Determination of the total flavonoid content (TFC)

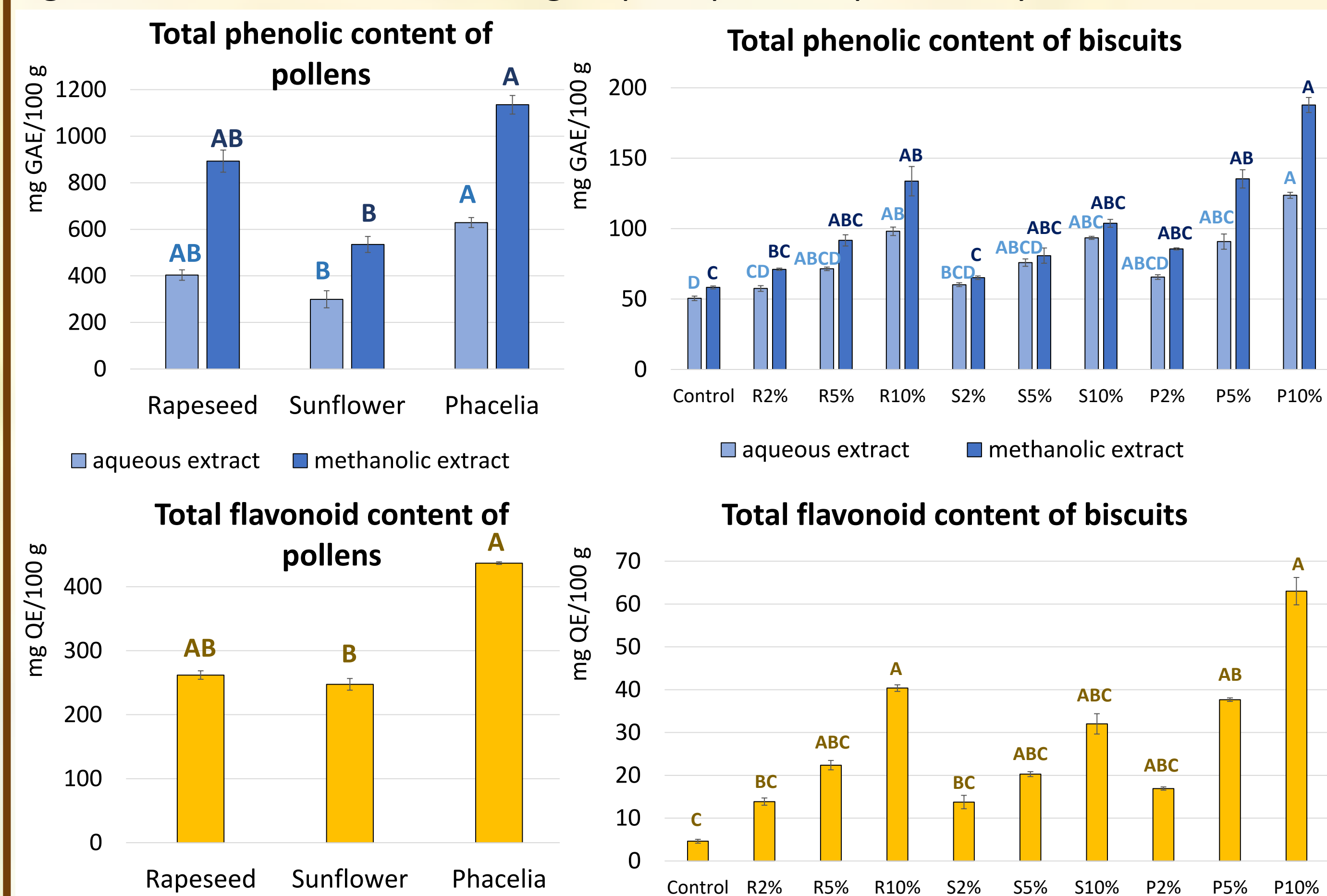
The total flavonoid content of the pollens and biscuits were determined by the method proposed by Woisky & Salatino (1998). Aluminium chloride was used for preparing the solutions, the absorbances of which were determined spectrophotometrically at 415 nm. Results were expressed as mg QE (quercetin equivalent)/100 g sample.

Statistical analysis

Measurements were conducted in five replicates. Kruskal–Wallis nonparametric test was applied to calculate the exact p value ($\alpha = 0.05$), and Dunn's pairwise procedure (post hoc test) was used with Bonferroni correction.

Results and discussion

Results of the TPC and TFC determinations of biscuits and pollens used for fortification are presented in the diagrams below. Different letters indicate significant differences between groups at $p < 0.05$ probability level.



Our results confirm that bee pollen is rich in antioxidants, since the TPC of the tested samples was more hundred mg GAE/100 g in each case. The methanolic extracts of pollens resulted in approximately two times higher TPC values compared to the aqueous extracts. Among the tested samples, phacelia pollen was the most significant source of polyphenols. The total flavonoid content of the pollens varied between 248 and 437 mg QE/100 g. Sunflower pollen showed the highest, while rapeseed pollen had the lowest TFC:TPC ratio.

In the case of biscuits, smaller differences were observed between the total phenolic contents of aqueous and methanolic extracts. The TPC of aqueous extracts varied between 51 (control) and 124 mg GAE/100 g (10 % fortification with phacelia pollen), while the TPC of the methanolic extracts were between 58 and 189 mg GAE/100 g. The higher the level of fortification was, the higher the TFC:TPC ratio was, which indicates that pollens contained more flavonoids than the other ingredients of the biscuits. Fortification of 10% with each pollen or 5% with phacelia pollen resulted in a statistically significant increase of TPC in the aqueous extracts of biscuits. In the case of methanolic extracts, fortification of 5% with phacelia pollen and 10% with rapeseed pollen were sufficient for statistically significant increase of TPC and TFC.

Conclusions

Based on our results, honeybee-collected pollens of rapeseed, sunflower and phacelia are rich in phenolic components and are suitable for the fortification of bakery goods. Results suggest that biscuits enriched with phacelia pollen contain the highest amount of polyphenols, nevertheless, further investigations are required in order to evaluate other nutritional properties, physical parameters and sensory characteristics of the biscuits.

References

- AACC (1980). AACC Approved Method 10-50D: Baking Quality of Cookie Flour
- Kieliszek, M., Piwowarek, K., Kot, A. M., Błażej, S., Chlebowska-Śmigiel, A., Wolska, I. (2018). Pollen and bee bread as new health-oriented products: A review. *Trends in Food Science and Technology*, 71, 170-180. <https://doi.org/10.1016/j.tifs.2017.10.021>
- Kostić, A. Ž., Milinčić, D. D., Barać, M. B., Shariati, M. A., Tesić, Ž. L., Pesić, M. B. (2020). The application of pollen as a functional food and feed ingredient - The present and perspectives. *Biomolecules*, 10, 84. <https://doi.org/10.3390/biom10010084>
- Singleton, V. L. & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144-158.
- Woisky, R. G., & Salatino, A. (1998). Analysis of propolis: Some parameters and procedures for chemical quality control. *Journal of Apicultural Research*, 37, 99-105.

Acknowledgements

This research was supported by the National Research, Development and Innovation Office of Hungary (OTKA, contracts No. 135700). The authors acknowledge the Hungarian University of Agriculture and Life Sciences's Doctoral School of Food Science for the support in this study.