

Title of the doctoral dissertation: Analysis of the chemical composition and biological activity of drone brood preparations and the possibility of their use in the design of dietary supplements

Summary

Drone brood is a less-known and studied bee product, often treated as waste by beekeepers, it is sometimes used as a natural way to combat varroa. Due to its unique chemical composition in Eastern European and Asian countries, it is valued and used for the prevention/treatment of many diseases, including male infertility and menopausal disorders in women.

The purpose of this study was to analyze the chemical composition and biological activity of manufactured drone brood preparations and the possibility of their use in the design of dietary supplements. For the first time, the variation of the composition depending on the stage of development and apiary origin was analyzed. Due to the low durability of the material, an effective method of fixing it was sought. The fixed brood was used to design an innovative dietary supplement and its bioavailability was evaluated by simulated enzymatic digestion.

The study was conducted for drone brood obtained at three stages of development from three Subcarpathian apiaries. Immediately after collection, the material was homogenized and stored frozen until analysis. The following parameters were analyzed: testosterone and estradiol content (by ELISA), antioxidant activity and profile of polyphenolic compounds (HPTLC), protein content and protein profile (SDS-PAGE), enzymatic activity (diastase, other glycosidases) and mineral content (ICP-OES).

The study showed that drone brood regardless of developmental stage (day 7, 11 and 14) is a valuable source of many components, and with the progress of development, an increase in testosterone and protein content and an enrichment of the protein profile were observed. Higher antioxidant activity was found at earlier stages of development (day 7-11), with hydrophilic antioxidants, mainly phenolic acids as well as flavonoids, being the dominant fraction. It was shown that despite similarities in the composition of drone brood and royal jelly,

measurements of the activity of marker acid glycosidases (N-acetyl-hexosaminidase and α -glucosidase) and iron content make it possible to clearly distinguish between these materials.

Fixation of drone brood by freeze-drying preserved the antioxidant potential of the brood and the total content of polyphenolic compounds (about 10% loss). Fixation of frozen brood (maximum addition of 4%) allowed full preservation of its antioxidant properties during 6-month storage at room temperature. Honey enriched with drone brood is a convenient form of introducing small doses of brood into the diet. Increasing the proportion of frozen brood in honey results in its fermentation, such a limitation does not occur when freeze-dried brood as a honey additive was used.

Freeze-dried drone brood was used to prepare an innovative two-ingredient dietary supplement in a convenient-to-use capsule form. The preparation was enriched in calcium, comparatively using calcium carbonate (inorganic form) and ground eggshells (organic). The bioavailability of selected components (hormones, calcium, protein, antioxidants as well as polyphenols) was evaluated using simulated *in vitro* digestion. It was shown that in the presence of calcium, brood hormones, unlike polyphenols, are better bioavailable in the intestine, and protein digestibility also increases. Evaluation of the daily intake of bioactive components from the designed supplements confirmed the safety of their use.

The conducted research provided new important information regarding the technology of obtaining drone brood with optimal bioactivity and the favorable way of its fixation. The designed supplement based on natural ingredients may find application in the prevention or treatment of osteoporosis, but its effectiveness should be confirmed in *in vivo* studies.