

The influence of the sweet clover (*Melilotus albus*) addition on the content of the biologically active compounds in nectar honeys

Summary:

Honey enriched with herbs are a new functional product available on the domestic market, which are an interesting object of scientific research. The addition of herbs to honey allows to enriched it with bioactive compounds from plant materials. Such products may compete with varietal honeys of well-known therapeutic properties, like sweet clover honey. Sweet clover honey can be obtained from the yellow sweet clover (*Melilotus officinalis*), a valued herbal plant used, inter alia in the treatment of thrombotic vein inflammation and varicose veins, or white sweet clover (*Melilotus albus*) cultivated in Poland for beekeeping purposes due to its high honey yield.

The aim of the study was to evaluate the suitability of white sweet clover as an addition that enriches nectar honey with bioactive compounds. Two varieties of honey have been used for enrichment: rapeseed honey and linden honey. The chemical composition of the plant material (fresh and dried flowers and leaves, as well as dry extracts) was determined for both species (white/yellow) and cultivated forms of white sweet clover (annual/ biennial). The properties of honey with different additions of sweet clover were analysed in comparison to natural sweet clover honey. The profile and content of phenolic compounds (HPLC-DAD method), volatile compounds profile (GC-MS method), protein profile (Native PAGE and SDS-PAGE electrophoresis) and antioxidant activity (FRAP assay, DPPH assay and total phenolic compounds content) were compared. The biological activity of the obtained enriched honeys was tested by *in vitro* method using intestinal epithelial cells IPEC-1 (MTS test and RTCA xCELLigence system) and in the platelet aggregation inhibition test. Their antimicrobial activity was also assessed.

It was found that high- quality sweet clover honey is difficult to obtain on the domestic market. The pollen analysis showed numerous cases of adulteration related to the wrong declaration of the variety by the beekeeper. As specific chemical markers of this type of honey a coumarin, kaempferol and naringenin have been recognized. However, high variation in phenolic compound profiles depending on geographic origin and / or harvest year has been

shown. The other analysed physicochemical parameters and protein profile did not allow to distinguishing sweet clover honey from other nectar honeys.

It has been proven that white sweet clover, especially the biennial form, is an equally valuable source of bioactive compounds (coumarin, flavonoids and phenolic acids) as yellow sweet clover. The sweet clover flowers contained more polyphenolic compounds than the leaves. However, the leaves exhibited higher antioxidant activity. Moreover, it has been found that the content of phenolic compounds varies from different growth stages of plant. The highest content of a coumarin and the other phenolic compound were determined in the leaves at the stage: before budding (the young leaves).

It was established that dried flowers, leaves and dry extract obtained from them can be used to enrich the honey. The addition of 1% of dried herbs gives an optimal enrichment, mainly in coumarin and hyperoside. In the case of 0,1 % addition of the dry extract (equivalent to 1% of the amount of dried), a much lower level of enrichment was found, which indicating the loss of bioactive compounds, especially a volatile coumarin during the production of the dry extract. The comparison between two used varieties of honey showed that rapeseed honey is better for the enrichment because promotes an effective maceration and provides a more favourable consistency of the final product. In the organoleptic evaluation, the honeys enriched with the dry extract of sweet clover flowers were the most preferable.

Based on the *in vitro* test results it was concluded that the enrichment of nectar honey with plant addition improves the biological activity of the product. The enrichment of rape honey with sweet clover increased its antibacterial activity, especially against Gram-positive bacteria compared to rape honey. The tests on cell lines showed that honey with additions may have a protective effect on intestinal epithelial cells. Moreover, the honey with the addition of sweet clover flower showed the ability to inhibit platelet aggregation induced by chemical agonists (collagen and adenosine diphosphate), which may indicate a potential use of the product in the prevention of blood clotting. However, its usage requires *in vivo* tests on the safety and health effects of consumption.