Abstract

Innovative Method for Enhancing the Biological Activity of Honey †

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Honey is a natural product composed of more than 80% sugars, the main constituents being fructose and glucose. It also contains a small amount of bioactive compounds, such as polyphenols, which provide the biological activity of honey [1]. The aim of this study was to obtain honey enriched in polyphenols, having enhanced biological properties. This was based on the solubilization of phenolic compounds extracted from propolis in honey. We present preliminary results on the biological (antioxidant, antimicrobial and prebiotic) activities of honey enriched in polyphenols.

The polyphenols were extracted from propolis by ultrasound-assisted extraction with 75% ethanol solution and the ratio of substrate to solvent at 1:5, for 30 minutes at room temperature. The extract was solubilized in honey in an ultrasonic bath, mixed thoroughly, and the polyphenols were left to diffuse overnight. Honey enriched with polyphenols from propolis was tested and compared with simple honey in terms of antioxidant, antimicrobial and prebiotic effect. The antioxidant activity (AOC) of the samples was assayed using three spectrophotometric methods: radical scavenging activity (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid, ABTS, and α, α-diphenyl-β-picrylhydrazyl, DPPH) and reducing antioxidant power (Cupric reducing antioxidant capacity CUPRAC). The total phenolic content (TPC) was determined with Folin–Ciocalteu. The dried extract of propolis was analyzed with Fourier transform infrared (FTIR) spectra to assess the chemical functional groups of the extract. Honey mixed with the dried extract of propolis showed an almost four-fold increase in TPC, which was also reflected in the antioxidant activity determined by ABTS and DPPH.

The antimicrobial activity of the sample was performed by determining the minimum inhibitory concentration (MIC) of the growth and adhesion capacity to an inert substrate. Quantitative analysis of adhesion to the inert substrate of Salmonella enterica (Se) and Bacillus cereus (Bc) strains was performed by slim method using liquid medium (TSB) in 96-well plates using controls for sterility of the environment. The adherent biomass to the walls of a 96-well plate was evaluated by the micro-titration method that involves washing with physiological sterile water,
fixing with cold methanol, and staining with purple crystal. The optical density of the adhered biological material was visualized under a stereomicroscope. The adherent biomass was quantified by resuspending it in acetic acid and reading the absorbance at 490 nm. Honey enriched with polyphenol extract exhibited higher anti-adhesion compared to simple honey, but it showed higher inhibition of bacterial growth only in the case of B. cereus.

The prebiotic activity of the honey–extract mixture was evaluated on a strain of Lactobacillus sp. and compared to simple honey. Honey had a significant prebiotic effect by stimulating the growth of the Lactobacillus sp., while the polyphenol extract did not influence the prebiotic effect of honey. Further parameters such as short-chain fatty acid production and lipid peroxidation in the cell membrane will be analyzed and the effect on other probiotic strains will be tested.

Our preliminary results show that one can increase the biological activity of honey by enriching it in bioactive compounds from other sources, the activity of which is preserved in honey.

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