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Evaluation of phenolic content, antioxidant activity and sensory characteristics of Serbian honey-based product

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ABSTRACT

Serbian local honey-based product, consisting of a traditional basic honey with dried fruits added, is characterized in terms of antioxidant and sensory properties. Linden (L) and Homolje (H) honey with prunes (20, 30, and 40%, w/w) were examined in order to determine the total phenolics (TPh) and flavonoid (TFd) content, antioxidant activity, colour and sensory properties. TPh increased by 2.2 times for L40 and 2.4 times for H40. The increase in TFd was higher, approximately 4.5-fold for L40 and 5-fold for H40. The greatest increase of antioxidant activity was noted for superoxide anion and 2,2-diphenyl-1-picrylhydrazyl radicals, where EC_{50} of H40 decreased 4.5 and 4.3 times, respectively. The L^* value decreased with increasing the concentration of prunes in both honey types. L40 (L^* = 25.44) and H40 (L^* = 34.19) honey-based products were the darkest ones. The statistical analysis suggested that TPh and TFd were associated with antioxidant activity and colour of honeys and honey-based products. Obtained scores for individual sensory properties indicated very good quality of honey-based products.

1. Introduction

According to the Codex Alimentarius Comission (2001) honey is defined as "the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature" (Codex Alimentarius Commission, 2001). Honey is a highly concentrated solution of complex mixture of sugars of which fructose and glucose are the main contributors. Also, honey contains wide range of minor constituents including enzymatic and non-enzymatic antioxidants such as glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins (Lachman et al., 2010; Aljadi and Kamaruddin, 2004; Gheldof et al., 2002).

From the ancient times up to the present day honey is well known for its high nutritional and medicinal value. The prophylactic and therapeutic potential of honey on human health, such as cardioprotective, anti-carcinogenic, antimicrobial, immune-stimulant and antiinflammatory effects, can be attributed to the presence of various antioxidant compounds (Küçük et al., 2007; McKibben and Engeseth, 2002; Liu et al., 2013).

The plant species from which the nectar or the honeydew was collected, as well as climatic and environmental conditions, and processing methods strongly affect physicochemical, sensory and health-protective characteristics of honey (Cimpoiu et al., 2013; Liu et al., 2013). Although Serbia is a small country, differences in climate, soil and plants provide a solid base for the production of different types of honey. Accordingly, in addition to the usual types of honey (acacia, meadow, linden and chestnut honey), rare and unique species, derived from plants characteristic of a particular region of Serbia, are also produced. In Serbia, honey is usually used in the original, unprocessed form, as a thick liquid or crystallized form. Honey is often enriched with pollen, propolis, royal jelly, or other primary bee products without changing its composition, but enhancing the taste, nutritional and medicinal properties. Besides these types of honey, honey with nuts (walnut, almond and hazelnut) and dried fruits (prune and fig) is also prepared and consumed as a tasty dessert.

In view of the foregoing, our interest is focused on honey with dried fruits and, to the best of our knowledge, in our previous study (Tumbas et al., 2012) we were the first ones to report

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bioactivity of this kind of product. The aim and purpose of the present study was to evaluate total phenolic and flavonoid contents, colour and sensory characteristics, as well as antioxidant activity of two different Serbian honeys with prunes, by four different assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl ($^{\bullet}OH$) and superoxide anion ($O_2^{\bullet-}$) free radical scavenging assays and reducing power (RP). In addition, Pearson's analysis was carried out to find the relationship between all analyzed parameters.

2. Experimental

2.1. Chemicals and instruments

The chemicals used for these investigations were Folin–Ciocalteu reagent, crown ether (Fluka Chemical Co., Buchs, Switzerland), trichloroacetic acid, 2,2-diphenyl-1-pichrylhydrazyl, 5,5-dimethyl-1-pyroline-*N*-oxide (DMPO), KO_2, rutin and gallic acid (Sigma Chemical Co., St. Louis, Mo, USA). All other chemicals and reagents were of the highest analytical grade, obtained from J.T. Baker (Deventer, Holland). The total phenolic, flavonoid, DPPH free radical scavenging assays and reducing power were determined using an UV-1800 spectrophotometer (Schimadzu, Kyoto, Japan), while the antioxidant activity against reactive hydroxyl and superoxide anion radicals was evaluated by electron spin resonance (ESR) spectroscopy (Bruker 300E ESR spectrometer, Rheinstetten, Germany). The CIE $L^*a^*b^*$ colour coordinates were measured using MINOLTA Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan).

2.2. Honey, prune and honey-based products

The honey-based products (honey with prunes) are prepared from two Serbian honeys, monofloral linden (L) honey (obtained from the honeybee farm "Simonović", Belgrade, Serbia) and polyfloral Homolje (H) honey (obtained from the honeybee farm "Homoljemed", Žagubica, Serbia). Prunes (obtained from the producer "Tehno-Božići", Šabac, Serbia) were cut into four pieces and added to the honeys in mass concentrations (w/w) of 20% (L20 and H20), 30% (L30 and H30) and 40% (L40 and H40). Honey-based products were analyzed one month after the addition of prunes.

2.3. Total phenolic (TPh) and flavonoid (TFd) contents

Total phenolics were determined spectrophotometrically by the Folin–Ciocalteu method (Singleton et al., 1999). The content of total phenolics was expressed as mg of gallic acid equivalents per 100 g of honey or honey-based product (mg GAE/100 g).

Total flavonoids were measured by the aluminium chloride spectrophotometric assay (Zhishen et al., 1999). Total flavonoid content was expressed as mg of rutin equivalents per 100 g of honey or honey-based product (mg RE/100 g).

2.4. Reducing power

The solution of honey or honey-based product (10–140 mg) in 1 ml of distilled water or 1 ml of distilled water (blank) was mixed with 1 ml of phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide $K_3[Fe(CN)_6]$. The mixture was incubated at 50 °C for 20 min and then rapidly cooled. Following this, 1 ml of trichloroacetic acid (10%) was added and the mixture was then centrifuged at 3000 rpm for 10 min. An aliquot (2 ml) of the upper layer, mixed with 2 ml of distilled water and 0.4 ml of 0.1% $FeCl_3$, was left to stand for 10 min. The absorbance of the mixture was measured at 700 nm against the blank.

2.5. DPPH free radical scavenging assay

Honeys and honey-based products were dissolved in methanol, and 1.5 ml of each sample or 1.5 ml of methanol (blank) was mixed with 3 ml of DPPH radical in methanol (0.02 mg/ml). The range of investigated concentrations was 0.33–166.67 mg/ml. The mixtures were left for 15 min at room temperature and then the absorbances were measured at 517 nm against reference mixtures that were prepared in the similar manner, by replacing the DPPH radical solution with methanol. The ability of honeys or honey-based products to scavenge DPPH radicals, $SA_{DPPH^{\bullet}}$ value, was calculated using the following equation:

$$SA_{DPPH^{\bullet}}(\%) = 100 \times \frac{A_0 - A_x}{A_0}$$

where A_0 and A_x are the absorbances of the blank and the sample, respectively.

2.6. Hydroxyl radical scavenging activity

Hydroxyl radicals were generated in the Fenton reaction system obtained by mixing 200 μl of 112 mM DMPO, 200 μl of H_2O, 200 μl of 2 mM H_2O_2, and 2 μl of 30 mM Fe^{2+} (control). The influence of honey or honey-based product, at the range of concentrations 2.5–25.0 mg/ml, on the formation and stabilization of hydroxyl radicals was investigated by ESR spin trapping method. The ESR spectra were recorded after 2.5 min, with the following spectrometer settings: field modulation 100 kHz, modulation amplitude 0.226 G, receiver gain 5×10^5, time constant 80.72 ms, conversion time 327.68 ms, centre field 3440.00 G, sweep width 100.00 G, *x*-band frequency 9.64 GHz, power 20 mW and temperature 23 °C. Hydroxyl radical scavenging activity of honeys and honey based-samples ($SA^{\bullet}_{OH}$) was defined as:

$$SA^{\bullet}_{OH}(\%) = 100 \times \frac{h_0 - h_x}{h_0}$$

where h_0 and h_x are the heights of the second peak in the ESR spectrum of DMPO-OH spin adduct of the control and the sample, respectively.

2.7. Superoxide anion radical scavenging activity

Superoxide anion radicals were generated in the reaction system obtained by mixing 500 μl of dry dimethylsulphoxide (DMSO), 5 μl of KO_2/crown ether (10 mM/20 mM) prepared in dry DMSO and 5 μl of 2 M DMSO solution of DMPO as spin trap. The influence of honeys and honey-based products on the formation and transformation of superoxide anion radicals was obtained by adding the DMF solutions of samples to the superoxide anion reaction system at the range of concentrations 20–200 mg/ml. After that, the mixture was transferred to a quartz flat cell ER-160FT. The ESR spectra were recorded under the following conditions: field modulation 100 kHz, modulation amplitude 4.00 G, receiver gain 1×10^4, time constant 327.68 ms, conversion time 40.96 ms, centre field 3440.00 G, sweep width 100.00 G, X-band frequency 9.64 GHz, power 20 mW, temperature 23 °C. The superoxide anion radical scavenging activity ($SA_{O_2^{\bullet-}}$) of honeys and honey-based products was defined as:

$$SA_{O_2^{\bullet-}}(\%) = 100 \times \frac{h_0 - h_x}{h_0}$$

where h_0 and h_x are the heights of the second peak in the ESR spectrum of DMPO-OOH spin adduct of the control and the sample, respectively.