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Biochemical properties, antibacterial and cellular antioxidant activities of buckwheat honey in comparison to manuka honey

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ABSTRACT

The biochemical properties of buckwheat honey, including contents of sugars, proteins, total phenols, methylglyoxal (MGO), minerals and phenolic compounds, were determined in comparison with those of manuka honey. Buckwheat honey has higher contents of sugars, proteins and total phenols but a lower content of MGO than manuka honey. Buckwheat honey contains abundant minerals involved in a number of vital functions of the human body as does manuka honey, and has even higher contents of Fe, Mn and Zn. In buckwheat honey, p-hydroxybenzoic acid, chlorogenic acid and p-coumaric acid are the dominant phenolic compounds. Moreover, the antibacterial and cellular antioxidant activities of buckwheat honey were compared with those of manuka honey. Buckwheat honey exhibits antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa,* comparable with manuka honey, and the cellular antioxidant activity of buckwheat honey is higher than that of manuka honey. Our results suggest that buckwheat honey has great nutritional and commercial potentials.

1. Introduction

As a natural food source containing abundant nutrients, honey is widely consumed because many studies have demonstrated its various beneficial biological activities, such as antioxidant, antibacterial, anti-browning, ACE-inhibitory and anti-inflammatory activities (Chang, Wang, Yang, Chen, & Song, 2011; Leon-Ruiz et al., 2013; Liu, Ye, Lin, Wang, & Peng, 2013). The major components of honey are sugars; however, proteins, minerals, phenolic compounds and other minor components also greatly contribute to its biological activities (Moniruzzaman, Sulaiman, Khalil, & Gan, 2013). The compositions and biological activities of honey vary, largely depending on the botanical and geographical origins (Alzahrani et al., 2012).

Buckwheat honey originates from the flowers of buckwheat (*Fagopyrum esculentum* Moehch). China is one of the main production regions of buckwheat honey in the world. Previous studies have demonstrated that buckwheat honey has antibacterial and antioxidant activities (Brudzynski, Abubaker, & Wang, 2012; Gheldof, Wang, & Engeseth, 2002; Zhou et al., 2012). However, due to its dark amber colour and strong pungent odour, buckwheat honey is not widely consumed. As a result, there have been rather limited research data reported about it.

Manuka honey, of New Zealand, which is characterized by its dark colour, is well known for its excellent antibacterial and antioxidant activities (Alvarez-Suarez et al., 2016; Boateng & Diunase, 2015; Stephens et al., 2010). It was reported that dark-coloured honey has a higher phenolic content (Alvarez-Suarez et al., 2010; Moniruzzaman et al., 2013), and many studies have shown that honey with a high phenolic content exhibits potent antibacterial and antioxidant activities (Bertoncelj, Dobersek, Jamnik, & Golob, 2007; da Silva et al., 2013; Ferreira, Aires, Barreira, & Estevinho, 2009; Sousa et al., 2016). Buckwheat honey also has a dark colour, and thus it may possess a high phenolic content and have antibacterial and antioxidant activities comparable or even superior to manuka honey.

The physicochemical properties of honeys from different countries have been extensively studied (Ozcan & Olmez, 2014; Silva, Videira, Monteiro, Valentao, & Andrade, 2009; Can et al., 2015). However, to measure the nutritional value and health benefits of honey, the analysis of biochemical components is more effective than physicochemical determinations (Saxena, Gautam, & Sharma, 2010). So far, very few studies have been focussed on the biochemical properties of buckwheat honey. For evaluating the antioxidant activity of honey, chemical-based methods, such as ferric reducing power assay, DPPH-free radical-scavenging assay, ABTS radical-scavenging assay and total antioxidant

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activity, have been commonly used (Bueno-Costa et al., 2016; Can et al., 2015; Gasic et al., 2014). However, none of these methods takes into account the bioavailability, uptake, and metabolism of the antioxidant. Cell-based antioxidant activity assay has a great advantage over chemical-based methods when used to evaluate the potential bioactivity of antioxidants under physiological conditions (Li et al., 2016; Wolfe & Liu, 2007). To our knowledge, the cellular antioxidant activity of buckwheat honey has not yet been investigated.

The present study was aimed to systematically evaluate the biochemical properties, antibacterial and cellular antioxidant activities of buckwheat honey through a comparison with manuka honey. The results may help to improve the understanding of the nutritional and commercial values of buckwheat honey.

2. Materials and methods

2.1. Honey samples and chemical reagents

Buckwheat honey samples were collected from the agricultural farms in Chaoyang, Liaoning Province, China. Manuka honey (AAH 8+) was purchased from Airborne Honey Limited Company (Leeston, Canterbury, New Zealand). The standards, 2′,7′-dichlorofluorescein diacetate (DCFH-DA) and 2,2′-azobis (2-amidinopropane) dihydrochloride (ABAP) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methylglyoxal (MGO, 40% w/w) and *o*-phenylendiamine (OPD) were obtained from Aladdin (Shanghai, China). DMEM medium, foetal bovine serum (FBS) and Hanks' balanced salt solution (HBSS) were purchased from Gibco Life Technologies (Grand Island, NY, USA). Acetonitrile and methanol for HPLC analysis were of HPLC grade. All other chemicals and reagents were of analytical grade.

2.2. Biochemical analysis

2.2.1. Sugars

The sugars of the honey samples were analyzed using chromatographic methods. Honey (0.2 g) was dissolved in 5 ml of 60% acetonitrile solution. The dissolution was then filtered through a 0.45 μm syringe filter. Standards of fructose, glucose, sucrose and maltose were mixed with 60% acetonitrile solution and diluted to different concentrations (0.5–30 mg/ml) for preparing the calibration curve. The determination of sugars was conducted with a Waters e2695 high-performance liquid chromatography (HPLC) system equipped with ELSD. The separation was performed on a Phenomenex NH_2 column (4.6 × 250 mm, 5.0 μm), and the mobile phase was 80/20 acetonitrile/H_2O with a flow rate of 1.0 ml/min. The column was kept at 30 °C and the injection volume was 10 μl.

2.2.2. Protein

The protein content was determined by Bradford's method (1976). 10 g of honey were dissolved in 10 ml of distilled water and centrifuged for 15 min at 4800 r/min. The supernatant was collected and diluted to 25 ml. One millilitre of this sample was mixed with 5 ml of Coomassie Brilliant Blue G-250 reagent solution (50 mg G-250 dissolved in 25 ml 95% ethanol and 50 ml 85% phosphoric acid and then diluted to 500 ml). Ten min later, the absorbance was measured at 595 nm. The protein content was calculated using the standard curve of bovine serum albumin (BSA), (0–100 μg/ml).

2.2.3. Total phenols

The total phenolic content was determined by using the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). Honey (5 g) was diluted to 50 ml with distilled water. One millilitre of this honey solution was mixed with 1 ml of Folin–Ciocalteu reagent and then thoroughly mixed by vortexing. The solution was treated with 5 ml of 1 M sodium carbonate solution, and made up to 10 ml. The reaction mixture was further incubated at room temperature in the dark for 1 h. The absorbance was measured at 760 nm, and gallic acid was used as standard.

2.2.4. Methylglyoxal

The content of MGO was analyzed as the corresponding quinoxaline after derivatization with OPD, according to the method of Oelschlaegel et al. (2012) with minor modifications. Honey (1 g) was dissolved in 10 ml of bidistilled water. One millilitre of the honey solution was treated with 1 ml of a 6 g/l aqueous solution of OPD. The reaction was performed in the dark for at least 8 h at room temperature. MGO standards, ranging from 0 to 0.096 mg/ml, were reacted with OPD using the same method as for the honey samples. After membrane filtration (0.45 μm), 10 μl of the derivatization mixture was injected into a Waters e2695 HPLC system. The analytical column was a Thermo Hypersil GOLD C18 column (4.6 × 250 mm, 5.0 μm), which was maintained at 30 °C. The mobile phase A was 0.1% acetic acid in water and mobile phase B was methanol. The elution conditions were: 0–5 min 30% B, 10 min 90% B, 15 min 90% B, 16 min 30% B and 20 min 30% B at a flow rate of 1.0 ml/min.

2.2.5. Minerals

Prior to analysis, the honey samples were submitted to sequential microwave-assisted digestion. Honey samples (1 g) were digested with 3 ml of HNO_3 and 3 ml of H_2O_2 using a CEM Mars5 microwave digestion system. The digestion programmes were: 240 W 1 min, 360 W 3 min and 600 W 5 min. Analysis of minerals in the honey samples was conducted by inductively coupled plasma mass spectrometry (ICP-MS, Aglient 7700E).

2.2.6. Extraction of phenolic compounds

Phenolic compounds were extracted from honey, as described in previous studies (Kassim, Achoui, Mustafa, Mohd, & Yusoff, 2010) with minor modifications. Honey (300 g) was mixed with 1500 ml of hydrochloric acid solution (pH 2), which was stirred to achieve complete dissolution, and then filtered by vacuum suction to remove solid particles. The solution was mixed with 400 g of Amberlite XAD-2 resin and stirred by a magnetic stirrer for 60 min. The mixture was transferred to a glass column (50 × 5 cm) and the column was washed with 1500 ml of hydrochloric acid solution (pH 2) and then with 2000 ml of distilled water in order to remove the sugars and polar constituents. The phenolic compounds absorbed on the resin were eluted with 1000 ml of methanol and concentrated to dryness under reduced pressure in a rotary evaporator at 40 °C. The residue was dissolved in 20 ml of distilled water and extracted with 60 ml of ethyl acetate at least three times. The extracts were combined and the ethyl acetate was removed by a rotary evaporator. The residue was re-suspended in distilled water and lyophilized.

2.2.7. Analysis of phenolic compounds by HPLC

HPLC analysis was performed using a Waters e2695 HPLC system and a Thermo Hypersil GOLD C18 column (4.6 × 250 mm, 5.0 μm). The gradient elution programme was established by following the methodology reported by Pasini, Gardini, Marcazzan, and Caboni (2013). The mobile phase consisted of 1% aqueous acetic acid (solvent A) and acetonitrile (solvent B). The injection volume was 10 μl, and the flow rate of the mobile phase was 0.5 ml/min. The elution conditions were as follows (min, (% B)): 0 (5), 10 (12), 15 (16), 30 (20), 40 (30), 50 (35), 60 (50), 70 (95), 75 (5) and 80 (5).

The phenolic compounds were identified by comparing the retention times and UV-spectra with the standards. Fifteen kinds of standards were selected for comparison on the basis of phenolic compounds previously found in honeys. Calibration curves were obtained by plotting the peak areas against the concentration of standards. The phenolic compounds were quantified by interpolation of the peak areas against the calibration curves.