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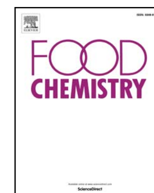

Biochemical properties, antibacterial and cellular antioxidant activities of buckwheat honey in comparison to manuka honey

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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.

2.3. Antibacterial activity

2.3.1. Agar well diffusion assay

Two bacterial strains were used for antibacterial assays, including *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative), which are common bacteria associated with wound infection. Suspensions of the bacteria were diluted in Muller Hinton (MH) broth to provide a cell density of 10^8 CFU/ml. 10 µl of this suspension were added to 100 ml of MH agar at 45 °C and poured into the plates. Prior to the addition of agar, five sterilized stainless steel borers were put into each plate to make wells (8 mm in diameter). The honey samples were prepared at different concentrations ranging from 2.5% to 90% (w/v) in MH broth and filtered through a 0.22 µm membrane filter. Each well was filled with honey solution and the plates were left at 4 °C for 8 h to allow the diffusion of the samples into the media, and subsequently incubated at 37 °C for 24 h. The inhibition zones, including the diameter of the well, were measured.

2.3.2. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the honey samples was determined by the broth dilution method in 96-well microplates. 90% (w/v) honey solution was prepared by weighing 9 g of honey and diluting to 10 ml, using MH broth, which was further diluted by the 2-fold serial dilution method. The bacterial cultures were diluted to the final concentration of 10^5 CFU/ml. 200 µl of tested honey solutions and 20 µl of bacterial suspension were dispensed into each well of the 96-well microplate. The microplates were incubated at 37 °C for 18 h and the optical density was determined at 600 nm. The lowest concentration of the honey inhibiting the bacterial growth was considered to be the MIC.

2.4. Cellular antioxidant activity (CAA)

2.4.1. Cell culture

HepG2 cell lines were obtained from the Cell Bank of Institute of the Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). HepG2 cells were cultured in DMEM medium (10% FBS and 1% penicillin/streptomycin (v/v)) and were maintained in a humidified incubator with atmosphere containing 5% CO_2 at 37 °C.

2.4.2. Determination of cytotoxicity

Cytotoxicity was measured by a methylene blue assay reported previously (Wolfe & Liu, 2007). Briefly, HepG2 cells were seeded in 96-well plates at a concentration of 4×10^4 cells/well. After 24 h of incubation at 37 °C, the cells were washed with PBS and treated with medium containing different concentrations of samples and incubated at 37 °C for 24 h. Control cells were treated only with medium. The number of cells was determined by methylene blue assay. The absorbance was measured using a microplate reader (Multiskan GO, Thermo Fisher, USA) at 570 nm. The samples with a $> 10\%$ reduction of absorbance compared with the control were considered to be cytotoxic.

2.4.3. Determination of CAA

The honey phenolic extracts in Section 2.2.6 were used for antioxidant assays. The CAA of honey extracts was measured by the method of Wolfe and Liu (2007) with some modifications. HepG2 cells were seeded at 4×10^4 cells/well on 96-well microplates and incubated for 24 h at 37 °C. Then, the medium was removed and replaced by 100 µl of growth medium containing honey extracts and 25 µM DCFH-DA. After 1 h of incubation, the cells were treated by PBS wash or no PBS wash. Subsequently, 600 µM ABAP dissolved in 100 µl of HBSS was added to the wells. The microplates were placed in a Multiskan GO microplate reader. Emission at 538 nm was measured with excitation at 485 nm every 5 min for 1 h.

The area under the fluorescence versus time curve was calculated as CAA value:

$$\text{CAA unit} = 100 - \left(\int SA / \int CA\right) \times 100$$

where $\int SA$ is the integrated area under the fluorescence versus time curve of the sample, and $\int CA$ is the integrated area under that of control. The median effective dose (EC_{50}) was determined for each honey sample from the median effect plot of log (f_a/f_u) versus log (dose), where f_a is the fraction affected and f_u is the fraction unaffected by the treatment (1-CAA unit).

2.5. Statistical analysis

All results were expressed as the means ± standard deviation (SD). Statistical analysis was performed using SPSS 22.0 Statistics. Differences between means at the 95% confidence level ($p < 0.05$) were considered as statistically significant.

3. Results and discussion

3.1. Biochemical properties

3.1.1. Sugars

Sugars are the main components in all honeys. We identified fructose, glucose, sucrose and maltose in the honey samples (Table 1). In both buckwheat honey and manuka honey, the monosaccharides fructose and glucose accounted for the largest proportion of sugars, and the content of fructose was higher than that of glucose. Pasini et al. (2013) reported that the content of fructose in buckwheat honey is 31–39% and that of glucose is 27–35%. The contents of fructose (36.4 ± 0.14%) and glucose (34.8 ± 0.35%) in buckwheat honey determined in this study are within this range. The content of fructose plus glucose of manuka honey (60.7 g/100 g) was similar to that reported by Moniruzzaman et al. (2013), which was lower than that of buckwheat honey (71.2 g/100 g). The sum of fructose and glucose in both honeys was higher than 60 g/100 g, which is in compliance with the Codex Standards (Codex Alimentarius Commission Standards, 2001). Sucrose was present in both types of honeys while a small quantity of maltose was found only in manuka honey. Low sucrose content indicates the conversion of sucrose to glucose and fructose, which is an important indicator of ripe honey. The levels of sucrose in buckwheat honey and manuka honey were below the maximum level (5%) required by the Codex Standards (Codex Alimentarius Commission Standards, 2001).

3.1.2. Protein content

The protein contents of the honey samples are shown in Table 1. The protein content of buckwheat honey was nearly three times that of manuka honey, and was also higher than that previously reported for the honey samples from Cuban, Romanian, Taiwan and some arid regions (Alvarez-Suarez et al., 2010; Cimpoiu, Hosu, Miclaus, & Puscas, 2013; Habib, Al Meqbali, Kamal, Souka, & Ibrahim, 2014; Liu et al., 2013). According to previous literatures, the protein contents of about 0.3 mg/g, 0.4–1 mg/g and 2 mg/g in honey are considered as low,

Table 1
Sugars, proteins, total phenols and MGO content of honeys.

Parameter	Buckwheat honey	Manuka honey
Fructose (g/100 g)	36.4 ± 0.14*	33.0 ± 0.17
Glucose (g/100 g)	34.8 ± 0.35*	27.8 ± 0.05
Sucrose (g/100 g)	0.60 ± 0.03*	0.98 ± 0.04
Maltose (g/100 g)	nd	0.42 ± 0.01
Protein (mg/g)	1.83 ± 0.01*	0.63 ± 0.01
Total phenols (mg/kg)	1498 ± 37.3*	561 ± 2.82
MGO (mg/kg)	4.61 ± 0.16*	351 ± 27.3

nd, not detected.
* $P < 0.05$, statistically significant in comparison with manuka honey.

medium and high, respectively (Alvarez-Suarez et al., 2010). Thus, buckwheat honey could be considered to have a high protein content (1.83 ± 0.01 mg/g), suggesting its high nutritional value.

3.1.3. Total phenolic content

The phenolic content of buckwheat honey was much higher than that of manuka honey (Table 1). Chang et al. (2011) determined the total phenolic content of sixteen floral honeys, and their results showed that the phenolic content ranged from 49 to 1008 mg/kg. Alzahrani et al. (2012) reported that manuka honey had the highest phenolic content (899 mg/kg) among the four floral honeys examined in their work. Liu et al. (2013) reported that the phenolic content of six floral honeys varied from 822 mg/kg to 307 mg/kg. To our knowledge, the phenolic content of buckwheat honey (1498 ± 37.3 mg/kg) was quite high among the floral honeys from different botanical and geographical origins. Many reports have shown a significant correlation between the phenolic content and antioxidant activity of honey (Bertoncelj et al., 2007; da Silva et al., 2013; Ferreira et al., 2009). The high phenolic value of buckwheat honey indicates that it has great antioxidant activity.

3.1.4. MGO

MGO was identified as the bioactive component responsible for the antibacterial activity of manuka honey (Mavric, Wittmann, Barth, & Henle, 2008; Stephens et al., 2010). Mavric et al. (2008) reported that the MGO content in manuka honey ranges from 38 mg/kg to 761 mg/kg. The MGO content of manuka honey measured in this study was 351 ± 27.3 mg/kg. It turned out that the MGO content of buckwheat honey was much lower than that of manuka honey (Table 1).

3.1.5. Minerals

The mineral content of honey is largely dependent on the floral and geographical origins, and has been considered as an important indicator of environmental pollution (Przybylowski & Wilczynska, 2001). Here, 17 minerals were investigated in honey samples and the results are summarized in Table 2. The most dominant minerals in buckwheat honey and manuka honey are Ca, Mg, Na and K, followed by Fe, Mn and Zn. Ca, Mg, Na and K were also found to be the major minerals in other honeys (Downey, Hussey, Kelly, Walshe, & Martin, 2005; Silva et al., 2009). These four minerals are macro-elements essential for human health. In buckwheat honey, the concentrations of Fe, Mn and Zn were significantly higher while that of K was lower compared with manuka honey. The levels of Ca, Mg, Mn and Zn in buckwheat honey were also higher than those detected in other honeys (Habib et al., 2014; Silva et al., 2009). Thus, the levels of these minerals could be considered as potential indices of buckwheat honey. Small amounts of B, Cu, Cr, Ni, Co, V and Mo were present in buckwheat honey. These micro-elements are involved in important functions of the human body. Our results show that buckwheat honey is rich in minerals. It has been reported that, when the content of minerals is higher, the colour of the honey is darker and the flavour is stronger (Gonzalez-Miret, Terrab, Hernanz, Fernandez-Recamales, & Heredia, 2005). Therefore, the dark colour and pungent odour of buckwheat honey may be attributed to its high content of minerals. The contents of toxic metals, including As, Cd and Pb, were below 0.02 mg/kg or not detectable, indicating that the honey samples were uncontaminated.

Table 2
Mineral contents in honey samples (mg/kg).

Minerals	Buckwheat honey	Manuka honey
Ca	1535 ± 186	1231 ± 103
Mg	316 ± 30.3	275 ± 26.3
Na	385 ± 35.3	347 ± 32.0
K	391 ± 12.2*	1228 ± 4.55
Fe	3.69 ± 0.40*	1.88 ± 0.49
Mn	6.77 ± 0.11*	2.15 ± 0.02
Zn	7.52 ± 1.25*	3.96 ± 0.41
B	4.22 ± 0.74	4.19 ± 0.49
Cu	0.25 ± 0.15	nd
Cr	0.29 ± 0.07	0.27 ± 0.09
Ni	0.21 ± 0.05	0.31 ± 0.04
Co	< 0.01 ± 0.00	< 0.01 ± 0.00
V	0.027 ± 0.006	0.019 ± 0.003
Mo	0.018 ± 0.008	< 0.01 ± 0.00
As	0.014 ± 0.009	< 0.01 ± 0.00
Cd	< 0.01 ± 0.00	< 0.01 ± 0.00
Pb	nd	nd

nd, not detected.
* P < 0.05, statistically significant in comparison with manuka honey.

Table 3
Contents of phenolic compounds in honey ethyl acetate extracts (μg/mg).

Compounds	Buckwheat honey	Manuka honey
Protocatechuic acid	1.17 ± 0.02*	0.14 ± 0.01
Chlorogenic acid	29.5 ± 0.97*	0.60 ± 0.01
p-Hydroxybenzoic acid	50.3 ± 1.12*	0.49 ± 0.01
Caffeic acid	4.06 ± 0.03*	1.06 ± 0.04
Syringic acid	0.05 ± 0.01	0.06 ± 0.01
p-Coumaric acid	11.0 ± 0.09*	0.14 ± 0.01
Ferulic acid	nd	nd
Isoferulic acid	nd	nd
Benzoic acid	8.46 ± 1.03*	0.51 ± 0.01
Quercetin	1.52 ± 0.03*	0.85 ± 0.02
Apigenin	0.46 ± 0.02*	0.14 ± 0.01
Kaempferol	1.47 ± 0.08*	0.29 ± 0.01
Isorhamnetin	nd	nd
Chrysin	0.31 ± 0.01*	0.71 ± 0.02
Galangin	1.24 ± 0.08*	2.40 ± 0.13

nd: not detected.
* P < 0.05, statistically significant in comparison with manuka honey.

3.1.6. Phenolic profile

The phenolic compounds of honeys were extracted by Amberlite XAD-2 resin, followed by purification with ethyl acetate. Nine phenolic acids (protocatechuic acid, chlorogenic acid, *p*-hydroxybenzoic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, isoferulic acid and benzoic acid) and six flavonoids (quercetin, apigenin, kaempferol, isorhamnetin, chrysin, galangin) were used as standards for the identification of phenolic compounds of honey. The contents of phenolic compounds in honey extracts are listed in Table 3. Among the phenolic compounds, *p*-hydroxybenzoic acid, chlorogenic acid and *p*-coumaric acid were at higher levels (50.3 ± 1.12, 29.5 ± 0.97 and 11.0 ± 0.09 μg/mg, respectively) in the buckwheat honey extracts, which were much higher than those in manuka honey. Previously, it was also reported that *p*-hydroxybenzoic acid and *p*-coumaric acids are the main phenolic acids in buckwheat honey (Pasini et al., 2013; Zhou et al., 2012), whereas the data for chlorogenic acid were not reported. The contents of benzoic acid and caffeic acid in buckwheat honey extracts were 4.06 ± 0.03 and 8.46 ± 1.03 μg/mg. Low levels of protocatechuic acid and syringic acid were present, while ferulic acid and isoferulic acid were not detectable. Flavonoids were present at relatively lower levels and isorhamnetin was not detectable in buckwheat honey extracts. Quercetin and kaempferol, the typical flavonoids found in other honeys, were also the main flavonoids found in buckwheat honey (Escuredo, Silva, Valentao, Seijo, & Andrade, 2012; Sousa et al., 2016). The content of total phenolic acids was much higher than that of total flavonoids in buckwheat honey. This result is in agreement with the previous report, which showed that dark-coloured honeys have more phenolic acids than flavonoids (Moniruzzaman et al., 2013). The total content of phenolic compounds in buckwheat honey extracts (110 μg/mg) was higher than that in manuka honey extracts (7.39 μg/mg), which may contribute to the antioxidant potential of buckwheat honey.

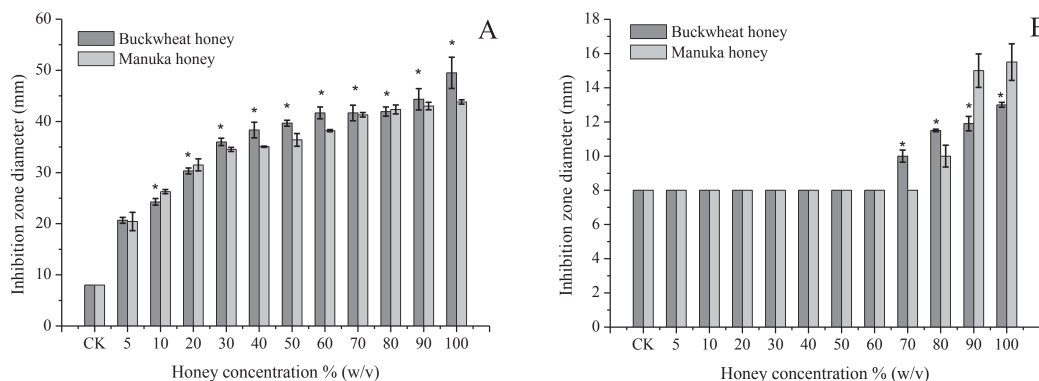

Fig. 1. Inhibition zone diameter (mm) of honeys at different concentrations (%, w/v) against *S. aureus* (A) and *P. aeruginosa* (B) in the agar-well diffusion test. *P < 0.05, statistically significant in comparison with manuka honey.

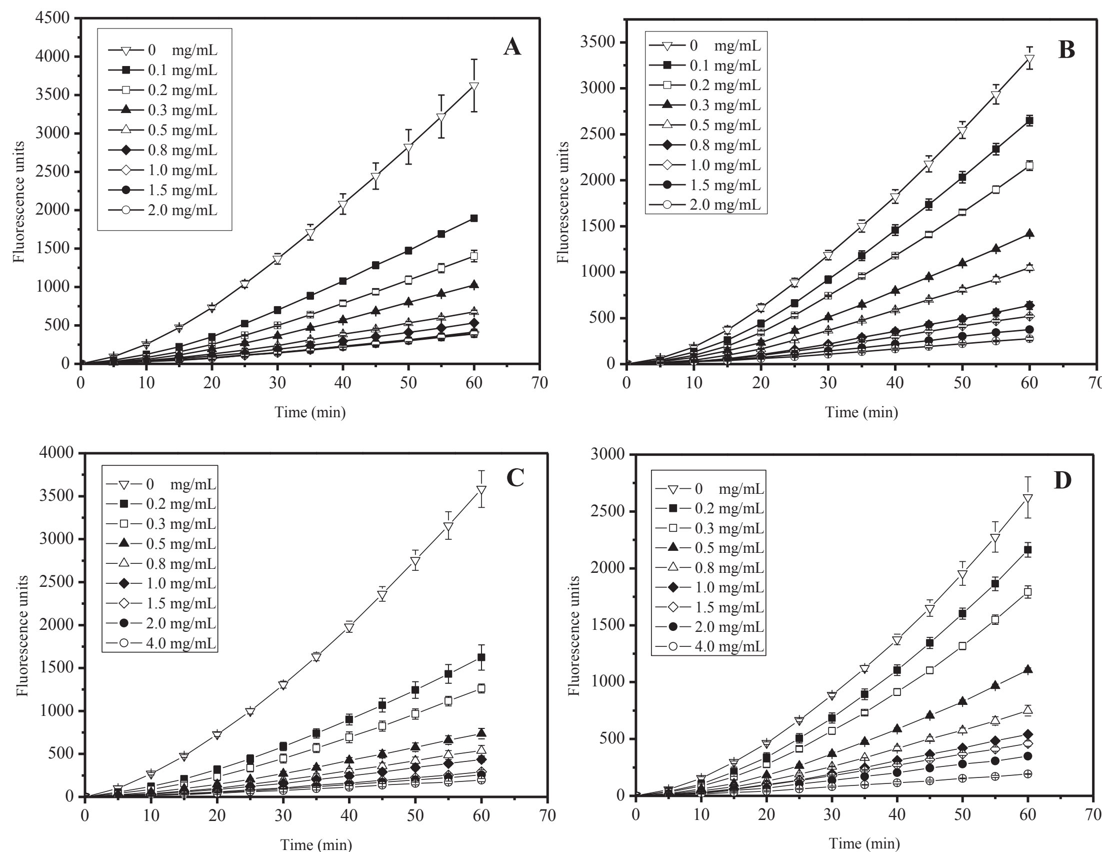

Fig. 2. Peroxyl radical-induced oxidation of DCFH to DCF in HepG2 cells and the inhibition of oxidation by buckwheat honey extracts (A, B), manuka honey extracts (C, D) over time, using the protocol involving no PBS wash between antioxidant and ABAP treatments (A, C) and the protocol with PBS wash (B, D).

Table 4
EC_{50} values for the inhibition of peroxyl radical-induced DCFH oxidation by honey extracts and their cytotoxic concentrations.

Honeys	EC_{50} (mg/ml)		Cytotoxicity (mg/ml)
	No PBS Wash	PBS Wash	
Buckwheat honey extracts	49.4 ± 6.35*	226 ± 15.9*	> 1.0
Manuka honey extracts	82.4 ± 11.1	324 ± 11.0	> 1.0

* P < 0.05, statistically significant in comparison with manuka honey.

3.2. Antibacterial activity

The agar well diffusion test was performed for the initial screening of antibacterial activity of honeys. The inhibition zone diameters of honey samples against *S. aureus* and *P. aeruginosa* are shown in Fig. 1. Both buckwheat honey and manuka honey inhibited the growth of *S. aureus* at all tested concentrations. The inhibition zone diameters of honey samples increased with increasing concentration in a dose-dependent manner. The inhibition zones of buckwheat honey at the concentrations of 30–100% were larger than those of manuka honey for *S. aureus*. However, for *P. aeruginosa*, both buckwheat honey and manuka honey exhibited no inhibition of its growth at the concentrations of 5–60%. At the concentrations of 70% and 80%, the inhibition zones of buckwheat honey were larger than those of manuka honey; but the opposite occurred when the concentration was 90% and 100%. Both buckwheat honey and manuka honey showed significantly higher antibacterial activity against *S. aureus* than against *P. aeruginosa* in the agar well diffusion test. The results are in agreement with previous studies, which showed that *S. aureus* is more sensitive to honeys than is *P. aeruginosa* (Anthimidou & Mossialos, 2013). As for the broth dilution test, equivalent MICs (22.5%) were found for buckwheat honey and

manuka honey against *S. aureus* and *P. aeruginosa*. It has been confirmed that the antibacterial activity of manuka honey is due to its high content of MGO (Stephens et al., 2010). Our results show that the MGO content in manuka honey is markedly higher than that in buckwheat honey. However, the antibacterial activity of buckwheat honey is comparable with that of manuka honey. Thus, the antibacterial activity of buckwheat honey may not be attributable to MGO. The previous study reported by Kwakman et al. (2010) showed that the major bactericidal factors in Revamil® medical-grade honey included its high sugar concentration, H_2O_2, low pH, MGO, and the cationic antimicrobial peptide (AMP) bee defensin-1. We guess that the components responsible for the antibacterial activity of buckwheat honey may be phenolic compounds, sugars, peptides and others.

3.3. Cellular antioxidant activity (CAA)

The antioxidant activity of honey samples was evaluated using a cell-based model of HepG2 cells. The CAA assay, which considers the bioavailability, cellular uptake, and distribution of bioactive compounds in the biologically relevant system, can determine the antioxidant activity more accurately than chemical-based methods (Wolfe & Liu, 2007). In this study, the CAA of buckwheat honey was determined for the first time. As phenolic compounds play an important role in the antioxidant activity of honey (da Silva et al., 2013; Ferreira et al., 2009), we used honey phenolic extracts for the determination of CAA. As shown in Fig. 2, the oxidation induced by proxyl radicals was inhibited by honey extracts, and the inhibitory effect was found to be concentration-dependent. As indicated by the fluorescence versus time curves, buckwheat honey extracts have a stronger inhibitory effect than have manuka honey extracts. The EC_{50} values of CAA for honey extracts and their cytotoxic concentrations are presented in Table 4. The EC_{50} value of buckwheat honey extracts was lower than that of manuka honey extracts, with or without a PBS wash, indicating that buckwheat honey possesses potent cellular antioxidant activity. The high content of phenolic compounds in buckwheat honey contributes to its antioxidant capacity. The EC_{50} value with no PBS wash was lower than that with a PBS wash, which is similar to the observations reported by other researchers (Li et al., 2016; Wen et al., 2016).

4. Conclusion

This study demonstrates that buckwheat honey has high contents of sugars, proteins, phenols and minerals, indicating that it is a good source of nutrients. The antibacterial activity of buckwheat honey is comparable with that of manuka honey. Furthermore, buckwheat honey possesses higher contents of phenolic compounds and better antioxidant capacity than does manuka honey. These results suggest buckwheat honey should be further investigated and more widely consumed because of its high nutritional value and important bioactive functions.

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The authors declare that there is no conflict of interests.

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