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Physicochemical and viscoelastic properties of honey from medicinal plants

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ABSTRACT

The present work investigated the physicochemical and structural properties of Tulsi, Alfalfa and two varieties of Manuka honey derived from medicinal plants. Chemical analysis yielded data on the content of reducing sugars (glucose and fructose) that dominate the honey matrix, and of the minor constituents of protein, phenols and flavonoids. Standard chemical assays were used to develop a database of water content, electrical conductivity, pH, ash content, visual appearance and colour intensity. Physicochemical characteristics were related to structural behaviour of the four honey types, as recorded by small-deformation dynamic oscillation in shear, micro- and modulated differential scanning calorimetry, wide angle X-ray diffraction and infrared spectroscopy. The preponderance of hydrogen bonds in intermolecular associations amongst monosaccharides in honey yields a semi-amorphous or semi-crystalline system. That allowed prediction of the calorimetric and mechanical glass transition temperatures that demarcate the passage from liquid-like to solid-like consistency at subzero temperatures.

1. Introduction

Honey is a natural sugar-saturated material used as food sweetener, complete food or medicinal supplement. Epidemiological studies reported protective and therapeutic effects of honey on overall health and well-being by improving the immune, antibacterial and antioxidation response extending to cardiovascular protection (Alvarez-Suarez, Gasparrini, Forbes-Hernández, Mazzoni, & Giampieri, 2014). It is made of sugar, mainly glucose and fructose up to 80% (w/w), and over 180 other components including proteins, free amino acids, essential minerals, vitamins, enzymes and phenolic phytochemicals (Alvarez-Suarez, Giampieri, & Battino, 2013). Phytochemicals are the main source of bioactivity and medicinal properties are transferred to honey through floral nectar and pollen collection by the bees (Alvarez-Suarez et al., 2010). They vary amongst plants to influence the level and diversity of bioactive compounds in honey but phenolic acids and flavonoids are the most abundant.

Tulsi plant (*Ocinum tenuiflorum* L.), Manuka tree (*Leptosperma scoparium*) and Alfalfa plant (*Medicago sativa*) have long been used in Indian traditional medicine as a source of bioactive molecules with therapeutic potential (Bora & Sharma, 2011). Literature reports that their pharmacological effects relate to anticancer, antiinflammation, hypolipidemia and cardioprotection, with the combination of nutritional and prophylactic properties promising long-term health benefits (Upadhyay et al., 2015).

To maximise health benefits and establish a solid platform of analytical information leading to application, there is a need to identify primarily the phenol and flavonoid contents in various types of commercially available honey (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005). Physicochemical characterization including water content, electrical conductivity, ash content, pH, visual colour and colour intensity, reducing sugar, and total protein can facilitate standardisation of honey bee products (Saxena, Gautam, & Sharma, 2010). Furthermore, quality attributes that relate to palatability *via* oral administration and topical treatment for infected wounds require fundamental understanding of the physical state of honey and its thermodynamic transition from liquid to solid-like behaviour as a function of environmental temperature (LeBail et al., 2003).

Honey is a high-solid material and should possess a characteristic glass transition temperature (T_g), which is a parameter widely used to predict, hence optimise the quality and stability of products during processing and subsequent storage. Above the respective T_g value, food products develop a rubbery and/or a melt state, with a considerable decrease in viscosity allowing for greater mobility. This outcome results in structural changes of the condensed matrix including collapse, stickiness, caking and fusion (Santivarangkna, Aschenbrenner, Kulozik, & Foerst, 2011). Below the glass transition temperature, systems enter the glassy region where molecular diffusion, leading to chemical, enzymatic and biological reactions, is limited (Roos, 2010). Recently, the concept of mechanical or network glass transition temperature has been introduced to complement estimates of the calorimetric T_g in glass forming matrices like honey as a function of

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temperature or timescale of observation (Kasapis, 2006).

Given the above, this study aims to examine the physicochemical and structural properties of various types of honey obtained from different medicinal plants known for antiinflammation and lowering cholesterol properties. Making available information from fundamental studies can facilitate development of product concepts, with honey being the main component, showing an increasingly likelihood of acceptance by the consumer.

2. Materials and methods

2.1. Materials

Four different types of honey, i.e. Tulsi (TUL), Alfafa (ALF) and Manuka (MH1 and MH2), were used in this study. The former is a monofloral honey produced from the nectar of Tulsi plants grown in the green house of RMIT University, Australia. Varieties ALF, MH1 and MH2 are from Pennsylvania (USA), New South Wales (Australia) and Warrandyte (New Zealand), respectively. They were stored in air-tight jars under dark ambient conditions and subjected to 40 °C heating for 5 min to provide a common baseline for all systems prior to experimentation. Chemicals and reagents used in this study were AR standard. Folin–Ciocalteu's phenol reagents (2 N), sodium carbonate (> 99.5%), absolute ethanol (> 99.8%) and gallic acid (> 97.9%) were purchased from Sigma-Aldrich Co (Sydney, Australia).

2.2. Methods

2.2.1. Standard physicochemical analyses

Triplicate measurements were taken from distinct batches for each of the four types of honey studied following the protocol of International Honey Commission (Bogdanov, Martin, & Lullmann, 2002). In doing so, water content and total soluble solids were measured with a Refracto 30GS (Mettler Toledo, Australia) and converted accordingly using the Chetaway Table. Electrical conductivity was measured on SevenCompact Conductivity Meter S230 (Mettler Toledo) at 20 °C in 20% (w/v) honey solution in Milli-Q water. Ash content was obtained by placing 5 g of honey in a crucible (Labec, Australia) and heating at 600 °C overnight in a muffle furnace. pH measurement of 10% (w/v) honey solution was performed following the method of Moniruzzaman, Khalil, Sulaiman, and Gan (2013).

Visual colour was assessed following a method described by Bertoncelj, Doberšek, Jamnik, and Golob (2007). A chromameter CR-400/410 (Konica Minolta, Australia) was used for CIE L^*, a^*, b^* measurements of our samples, where L^*: lightness, $-a^*$: greenness, a^*: redness and b^*: yellowness, as compared with the white tile background. Colour intensity of 50% (w/v) honey solution, which was filtered at 0.45 μm to remove any coarse particles, was measured as described by Beretta, Granata, Ferrero, Orioli, and Maffei Facino (2005). Spectrophotometric absorbance was taken at 450 nm using a Lambda 35 UV–vis spectrophotometer from Perkin Elmer (Waltham, USA).

2.2.2. Reducing sugars

Amounts of D-glucose and D-fructose in honey were determined with Megazyme's Assay Kit (K-SUFRG 06/14). D-glucose was determined by utilising hexokinase and glucose-6-phosphate without hydrolysing sucrose. D-fructose was determined subsequent to the determination of D-glucose following isomerisation with phosphoglucose isomerase. Samples were analysed in triplicate and the mean is expressed as g/100 g honey.

2.2.3. Protein content

This was determined with Thermo Scientific™ Coomassie (Bradford) Protein Assay Kit (23200). Twenty μl of 10% (w/v) honey solution were pipetted into a microplate. Then, 250 μl of Coomassie reagent were added and the plate was put in a shaker to incubate at ambient temperature for 10 min. Absorbance was measured at 595 nm with a Polar microplate reader, against a standard solution of bovine serum albumin (0–100 μg/ml) that reached linearity of $R^2 = 0.994$. Milli-Q water was used as blank, and each sample was analysed in triplicate, with the mean being expressed in mg/100 g honey.

2.2.4. Total phenolic content

This was determined with the Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999). Honey sample (5 g) was diluted to 50 ml with Milli-Q water and filtered through Whatman No. 1 paper. Solution (0.5 ml) was mixed with 2.5 ml of 0.2 N Folin-Ciocalteu reagent (Sigma-Aldrich, Australia) for 5 min and 2 ml of 75 g/l sodium carbonate was then added. Mixture was incubated at ambient temperature for 2 h before reading with a Lambda 35 UV–vis spectrophotometer. Absorbance was measured at 760 nm against an ethanol blank. Gallic acid was used to produce a standard curve from 0 to 100 mg/l and obtained linearity was $R^2 = 0.999$. All analyses were carried out in triplicate and the mean was expressed in mg of gallic acid equivalents (GAE)/100 g honey.

2.2.5. Fourier transform infrared spectroscopy

A spectrometer equipped with a MIRacleTM ZnSe single reflection ATR plate (Perkin-Elmer, Norwalk, USA) was used to record FTIR spectra for honey. In doing so, 0.5 g was placed onto the measuring plate and scanned forty times from 4000 to 650 cm^{-1} at a resolution of 4 cm^{-1} at ambient temperature.

2.2.6. X-ray diffraction analysis

Presence of crystal nuclei in honey was examined using a D4 Advanced Bruker AXS (Karlsruhe, Germany) attached with a Cu-Kα radiation source (l = 1.54 A°). Triplicate samples were loaded onto the measuring holder and covered with an X-ray film. Raw data were obtained within a 2θ range of 5–90° in the interval of 0.1° and subsequently analysed using Diffract.EVA version 4.1.1.

2.2.7. Modulated and microdifferential scanning calorimetry

First-order thermodynamic transitions of honey were detected with a Setaram VII microdifferential scanning calorimeter (Seturau, France). Hundred mg of honey were loaded into a standard Hastelloy cell and an identical-weight water sample was used as reference. They were equilibrated for 20 min at 20 °C, heated to 90 °C, and then cooled to 20 °C at 1 °C/min. Heat capacity measurements to determine the calorimetric glass transition temperature were conducted on Q2000 calorimeter (TA instruments, New Castle, USA), with nitrogen purge gas at a flow rate of 50 ml/min. Ten mg of honey were loaded into a hermetic aluminium pan and equilibrated for 20 min at 20 °C. Samples were cooled to −90 °C and heated up to 30 °C at 1 °C/min. Triplicate measurements were performed at modulation amplitude of 0.53 °C every 40 s.

2.2.8. Viscoelastic analysis

This was performed with ARG-2 controlled strain rheometer using a magnetic-thrust bearing technology (TA Instruments, New Castle, USA). The rheometer was connected to a liquid nitrogen system to achieve rapid and uniform cooling. Parallel plates of 5 mm diameter and 1 mm gap were used, with samples (0.5 g) being loaded at 15 °C and covered with silicone oil (BDH; 50 cS) to minimise moisture loss. They were equilibrated at 15 °C for 10 min, then cooled deeply within the subzero regime at 1 °C/min with a frequency of 1 rad/s and strain of 0.01%. Frequency sweeps of 0.1 to 100 rad/s were taken from −60 to −20 °C at intervals of three degree centigrade. Time-temperature superposition principle was implemented to generate the master curve of viscoelasticity for honey.

2.2.9. Statistical analysis

Statistical differences in Table 1, represented by letters in the same row for the physicochemical properties of all samples, were obtained