Food Chemistry 189 (2015) 52–59

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Discrimination of honey of different floral origins by a combination of various chemical parameters

Z. Jandrić [a,*], S.A. Haughey [b], R.D. Frew [a], K. McComb [c], P. Galvin-King [b], C.T. Elliott [b], A. Cannavan [a]

[a] Food and Environmental Protection Laboratory, FAO/IAEA Agriculture and Biotechnology Laboratories, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria
[b] Institute for Global Food Security, Queen's University Belfast, UK
[c] Oritain Global Limited, Invermay Agricultural Centre, Mosgiel, New Zealand

ARTICLE INFO

Article history:
Received 29 June 2014
Received in revised form 19 November 2014
Accepted 20 November 2014
Available online 7 February 2015

Keywords:
Honey
Discrimination
Metabolomics
Fingerprinting
Spectroscopy
Elemental profiling
Chemometrics

ABSTRACT

Honey is a high value food commodity with recognized nutraceutical properties. A primary driver of the value of honey is its floral origin. The feasibility of applying multivariate data analysis to various chemical parameters for the discrimination of honeys was explored. This approach was applied to four authentic honeys with different floral origins (rata, kamahi, clover and manuka) obtained from producers in New Zealand. Results from elemental profiling, stable isotope analysis, metabolomics (UPLC-QToF MS), and NIR, FT-IR, and Raman spectroscopic fingerprinting were analyzed. Orthogonal partial least square discriminant analysis (OPLS-DA) was used to determine which technique or combination of techniques provided the best classification and prediction abilities. Good prediction values were achieved using metabolite data (for all four honeys, $Q^2 = 0.52$; for manuka and clover, $Q^2 = 0.76$) and the trace element/isotopic data (for manuka and clover, $Q^2 = 0.65$), while the other chemical parameters showed promise when combined (for manuka and clover, $Q^2 = 0.43$).

1. Introduction

In recent years, there has been growing interest in verifying the floral origin of honey, especially in the characterization of unifloral honeys, which are often more valuable than polyfloral honeys. Certain types of unifloral honey have claimed or apparent benefits for human health and are used in the treatment of wounds and diseases because of their healing and antibacterial properties (Allen, Molan, & Reid, 1991; Robson, Dodd, & Thomas, 2009; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008; ftp://ftp.fao.org/docrep/fao/012/i0842e/i0842e00.pdf). Several studies have proven the antimicrobial, anti-inflammatory, antimutagenic, antitumor, antioxidative activities, as well as many other benefits for human health, of compounds such as phenolic acids and flavonoids (Cushnie & Lamb, 2005; Havsteen, 2002) that are components of these honeys. New Zealand manuka (*Leptospermum scoparium)* honey, for example, has been proven to have non-peroxide antibacterial activity (Allen et al., 1991). According to the Codex Alimentarius Standard for Honey (2001) and the European Commission Directive (2001), the use of botanical designation is allowed if a honey originates predominantly from the indicated floral source. Adulteration in terms of the dilution of honeys of high value floral origin with those of lower value has increased in recent years. Therefore, discrimination of honey by floral origin is of great importance.

Identification of the floral origin of honey is typically achieved by melissopalynological analysis based on pollen characterization, and presently is complemented by sensory and physico-chemical analysis. However, pollen identification requires a high degree of skill and in some cases gives erroneous results (Cavazza, Corradini, Musci, & Salvadeo, 2012; Molan, 1998), while the determination of physico-chemical parameters is broad and cannot be uniformly applied to all honey varieties. For example, a citrus honey was shown to have 18% pollen content from kiwi fruit, which does not produce nectar (Moar, 1985). This clearly shows that honeys can incorporate pollen that is unrelated to the nectar source. Therefore, there is an ongoing need to develop reliable, practical, and faster methods to discriminate between honeys of different floral origins.

Recently, there has been an increase in the number of analytical techniques applied to differentiate honeys, for example by the analysis of flavonoids (Chan et al., 2013; Trautvetter, Koelling-Speer, & Speer, 2009), amino acids (Keckeš et al., 2013; Rebane & Herodes, 2008), proteins (Wang et al., 2009), phenolic compounds (Cavazza et al., 2012; Stephens et al., 2010), honey volatiles

* Corresponding author. Tel.: +43 (1) 2600 28373; fax: +43 (1) 2600 28222.
E-mail address: z.jandric@iaea.org (Z. Jandrić).

http://dx.doi.org/10.1016/j.foodchem.2014.11.165
0308-8146/

(Senyuva et al., 2009; Stanimirova et al., 2010), carbohydrates (Senyuva et al., 2009), and trace elements (Marghitas et al., 2010). Vibrational spectroscopy techniques (NIR, FTIR, and NMR) combined with chemometrics have also been used for the determination of the floral origin of honey and development of classification models (Etzold & Lichtenberg-Kraag, 2008; Liang, Li, & Wu, 2013; Schievano, Stocchero, Morelato, Facchin, & Mammi, 2012). Typically, principal component analysis (PCA) was applied as a clustering method for preliminary evaluation of the data structure, followed by various classification methods such as linear discriminant analysis (LDA), discriminant partial least square regression (DPLS), soft independent modeling of class analogy (SIMCA), or back propagation neural networks (BPNN) (Etzold & Lichtenberg-Kraag, 2008; Liang et al., 2013; Schievano et al., 2012).

The high commercial value of some New Zealand honeys (*L. scoparium* (manuka), *Kunzea ericoides* (kanuka), and *Trifolium* spp. (clover)) has motivated intensive investigation by different research groups, their characterization being mostly based on targeted analysis of extractable organic components (Senanayake, 2006; Stephens et al., 2010; Yao et al., 2003).

To the best of our knowledge, most of the studies to date have employed a single technique (sensory, microscopic, chromatographic, or spectroscopic) applied to one group of samples. There have been no studies published on the use of combined techniques to gain additional information and to find out the most appropriate methodology for honey floral origin discrimination. The aim of this study was to explore the feasibility of a multivariate approach, applying multivariate analysis to the results of sample analysis by a number of physico-chemical techniques, for the discrimination of various honeys. This approach was applied to four authentic honeys with different floral origins (rata, kamahi, clover and manuka) obtained directly from honey producers in New Zealand. The data processing was performed on the results of chemical analyses using elemental profiling, stable isotope measurements, metabolomics (ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-QToF MS)), and vibrational spectroscopy (near-infrared (NIR), fourier transform infrared (FT-IR), and Raman spectroscopy) fingerprinting. Orthogonal partial least squares projections to latent structures discriminant analysis (OPLS-DA) was used to determine which technique or combination of techniques provided the best classification and prediction ability.

2. Materials and methods

2.1. Samples

Authentic honey samples ($n = 83$) were obtained directly from honey producers from a range of locations in both the North and South Islands of New Zealand. The data was divided into a training set (manuka (31), clover (12), kamahi (3), and rata (4)), which was used to build the models, and a test data set used for model validation (manuka (19), clover (5), kamahi (4), and rata (5)). Botanical origins were identified at source in New Zealand by melissopalynology; this technique is not able to distinguish manuka from kanuka honey so in this study these are classed together as manuka (Molan, 1998; Stephens & Molan, 2008). Honey samples were analyzed by UPLC-QToF MS in negative mode, IRMS, ICP-MS, NIR, FT-IR, and Raman spectroscopy.

2.2. Analytical methods

The analytical methods applied were previously described in the scientific literature. The methods are described briefly below, with references to the published papers where full details can be found.

2.2.1. Metabolomics using UPLC-QToF MS

For UPLC-QToF MS analysis, experiments were performed on a Waters ACQUITY™ UPLC™ system connected to Xevo G2 Q-ToF MS equipped with an electrospray ionization source (Waters Corp., Milford, MA, USA). The chromatographic and instrumental conditions (UPLC-QToF MS) used were as described by Jandric et al. (2014). Replicate injections ($n = 4$) were used in a random sequence. Honey samples (0.5 g) were diluted with 10 mL 1% of formic acid in methanol/water (1/1, v:v), shaken, sonicated (20 min), centrifuged using a high speed centrifuge (Sigma–Aldrich, St. Louis, MO, USA) and filtered (0.25 μm) before injection.

2.2.2. IRMS

The carbon and hydrogen isotopic ratios were determined following the procedure described in Ehtesham, Hayman, McComb, Van Hale, and Frew (2013) with minor modification required for the honey matrix as follows. A mass of 0.6 ± 0.1 mg of each honey sample was measured into tin capsules, in duplicate, before vacuum drying for 12 h. Carbon isotopes were assayed by combustion of the whole material to CO_2 gas in a Carlo Erba NA1500 elemental analyzer (CE Instruments, Milan, Italy) in an oxygen pulse. The isotopic composition of the sample gases was measured by a Delta Advantage isotope ratio mass spectrometer (Thermo-Finnigan, Bremen, Germany) operating in continuous flow mode. Raw delta values were normalized and reported against the international scales for carbon and nitrogen, VPDB and AIR, respectively. Normalization was made by three-point calibration with two glutamic acid international reference materials and a laboratory EDTA (Elemental Microanalysis Ltd., Devon, UK) standard for carbon (USGS-40 = −26.39‰, USGS-41 = 37.63‰, EDTA = −38.52). Time-based drift correction was calculated from the laboratory standard analyzed at regular intervals with the samples. Analytical precision based on the replicate analyses of the QC standard (EDTA, $n = 12$) was 0.2‰ for $\delta^{13}C$.

For δ^2H analysis, a mass of 0.5 ± 0.1 mg of each honey sample was measured into silver foil capsules, in triplicate, before vacuum drying for 5 days to remove water from the samples. No attempt was made to account for the exchangeable hydrogen so the δ^2H data are comparable within this dataset but not necessarily comparable with samples run in other laboratories. Total hydrogen in the solid material was assayed by pyrolysis over glassy carbon at 1450 °C to hydrogen gas in a Thermal Conversion/Elemental Analyzer, TC/EA (Thermo, Bremen, Germany). Analysis of the hydrogen gas pulse was undertaken using a Thermo 'Delta V' IRMS (Thermo, Bremen, Germany), running in continuous flow mode. Raw Isotope values were drift corrected where necessary, and normalized to international scales using the IAEA reference material IAEA-CH7 ($\delta^2H = -100.3‰$) which was analyzed with the samples.

2.2.3. ICP-MS

Standard methods were used as previously published (Caroli, Forte, Iamiceli, & Galoppi, 1999; Fernández-Torres et al., 2005).

A mass of approximately 0.5 g of each collected honey sample was accurately weighed into PTFE pressure vessels (CEM) in triplicate. To each vessel 5 mL of q-HNO_3 was added and the stopper and cap applied. The vessels were left 1–2 h for pre-digestion before microwave digestion in a Microwave Assisted Reaction System (MARS) (CEM, Matthews, NC, USA). Nine samples in triplicate, two reference materials in triplicate and four blanks were run as an analytical batch. The reference materials used during the analysis were a certified corn meal reference material CRM-CM-A (High-Purity Standards, Charleston, SC, USA) and a bulk clover honey sample QCCH that were run to provide continuity between