Food Chemistry 192 (2016) 1006–1014

Contents lists available at ScienceDirect

Food Chemistry

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

Fluorescence markers in some New Zealand honeys

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ARTICLE INFO

Article history:
Received 24 March 2015
Received in revised form 21 July 2015
Accepted 23 July 2015
Available online 26 July 2015

Keywords:
Fluorescence
Manuka (*Leptospermum scoparium*)
Kanuka (*Kunzea ericoides*)
Honey
Nectar

ABSTRACT

The fluorescence characteristics of various New Zealand honeys were investigated to establish if this technique might detect signatures unique to manuka (*Leptospermum scoparium*) and kanuka (*Kunzea ericoides*) honeys. We found unique fluorescence profiles for these honeys which distinguished them from other New Zealand honey floral types. Two excitation–emission (ex–em) marker wavelengths each for manuka and kanuka honeys were identified; manuka honey at 270–365 (MM1) and 330–470 (MM2) nm and kanuka honey at 275–305 (KM1) and 445–525 (KM2) nm. Dilution of manuka and kanuka honeys with other honey types that did not possess these fluorescence profiles resulted in a proportional reduction in fluorescence signal of the honeys at the marker wavelengths. By comparison, rewarewa (*Knightia excelsa*), kamahi (*Weinmannia racemosa*), and clover (*Trifolium* spp.) honeys did not exhibit unique fluorescence patterns. These findings suggests that a fluorescence-based screening approach has potential utility for determining the monoflorality status of manuka and kanuka honeys.

1. Introduction

Honey is a complex and supersaturated sugar solution comprising approximately 80% sugars and a unique combination of other compounds suspended in water (White & Doner, 1980). The sugar component of honey is comprised principally of the monosaccharides, fructose and glucose. The non-sugar proportion comprises a range of plant- or bee-derived compounds such as organic acids, proteins, amino acids, enzymes, pollen, pigments, mineral salts, wax, and plant secondary metabolites (Anklam, 1998; White & Doner, 1980). The chemical composition of honey varies between honey floral types but may also be influenced by geographical origin, climate (Anklam, 1998), honey processing and age (Stephens et al., 2015).

A honey can be monofloral or polyfloral in origin depending on whether it is derived from one or several plant species. According to international food standards, in order for a honey to be labelled with floral origin, it must originate wholly or predominantly from a particular floral source and display the corresponding organoleptic, physico-chemical, and microscopic properties (Codex Alimentarius Commission., 2001). It is generally accepted that honey produced in a natural environment containing mixed plant species is never monofloral as it is impossible to control honey bee behaviour in the forage field (Winston, 1987). It is therefore difficult to produce scientifically pure monofloral honeys. The co-existence of numerous floral species that produce surplus nectar in the same geographical region and flower together are exemplified by the *Leptospermum scoparium* and *Kunzea ericoides* populations in New Zealand (Stephens et al., 2010).

The floral origin of honey is a major determinant of premium value. Monofloral honeys typically command a higher value than polyfloral honeys as they exhibit distinct flavour and quality attributes that are not present to the same extent in the polyfloral types. Certain monofloral honey types also have a greater retail value than others. The New Zealand manuka (*L. scoparium*) and Yemen sidr (*Ziziphus spina-christi*) honeys are examples of honeys traded at a premium worldwide due to their reported health benefits. Where a particular floral source commands a higher market value, an incentive exists to attribute that nectar source over others. Consumer expectation for true-to-label honeys as well as a concern over the authenticity of New Zealand premium honey products have identified a need for reliable and reproducible methods for determining honey monoflorality.

The current standard reference method to ascertain honey floral types is melissopalynology based on microscopic identification and quantification of pollen composition (Jones & Bryant, 1992; Louveaux, Maurizio, & Vorwohl, 1978). However, some pollen grains are difficult to identify accurately, and in the case of New Zealand manuka (*L. scoparium*) and kanuka (*K. ericoides*) honeys,

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http://dx.doi.org/10.1016/j.foodchem.2015.07.118
0308-8146/

the pollens of these species are virtually indistinguishable in a honey medium due to their close resemblance (Moar, 1985). Furthermore, pollen count also does not always accurately represent nectar contribution of a particular floral species in honey because the floral structure of certain plant species, such as the New Zealand rewarewa (*Knightia excels*a), allows honey bees to collect nectar without transferring the pollen grains (Moar, 1985). Physico-chemical and sensory analysis are routinely used in conjunction with melissopalynology for characterisation of honey floral origin (Bogdanov, Ruoff, & Persano Oddo, 2004; Piana et al., 2004).

Some honey types are also characterised by the presence of unique chemical compounds enabling chemical fingerprinting to determine floral origin. Manuka honey, for instance, is characterised by the presence of the dihydroxyacetone (DHA) and methylglyoxal (MGO) which are unique to the *Leptospermum* genus (Stephens et al., 2010; Windsor, Pappalardo, Brooks, Williams, & Manley-Harris, 2012) and also elevated concentration of the phenolic compound 2-methoxybenzoic acid (Beitlich, Koelling-Speer, Oelschlaegel, & Speer, 2014; Senanayake, 2006; Stephens et al., 2010). More recently, a novel glycoside of methyl syringate, leptosperin, has been proposed as a potential chemical marker for manuka honey (Fearnley et al., 2012; Kato et al., 2012, 2014; Oelschlaegel et al., 2012). Kanuka honey appeared to be characterised by elevated concentrations of 4-methoxyphenyllactic acid and methyl syringate (Beitlich et al., 2014; Senanayake, 2006; Stephens et al., 2010). Pasture and other pale-coloured honey types, in contrast, contain low level of phenolic and polyphenolic compounds (Stephens et al., 2010; Tan, Holland, Wilkins, & Molan, 1988).

Application of fluorescence spectroscopy in food analysis is becoming increasingly popular and has been demonstrated to be capable of characterising foods such as milk (Kulmyrzaev & Dufour, 2002; Kulmyrzaev, Levieux, & Dufour, 2005), cheeses (Karoui, Bosset, Mazerolles, Kulmyrzaev, & Dufour, 2005; Karoui et al., 2004), cereals (Karoui, Cartaud, & Dufour, 2006), and honeys (Aitkenhead, Rosendale, Schlothauer, & Stephens, 2014; Gebala & Przybylowski, 2010; Ghosh, Verma, Majumder, & Gupta, 2005; Karoui, Dufour, Bosset, & De Baerdemaeker, 2007; Lenhardt, Bro, Zeković, Dramićanin, & Dramićanin, 2015; Ruoff et al., 2006).

The fluorescence property of honey is attributed to the presence of phenolic and polyphenolic compounds (Aitkenhead et al., 2014; Gebala & Przybylowski, 2010; Ghosh et al., 2005; Karoui et al., 2007; Lenhardt et al., 2015; Ruoff et al., 2006), aromatic amino acids (Karoui et al., 2007; Lenhardt et al., 2015; Ruoff et al., 2006), and Maillard reaction products (Karoui et al., 2007; Lenhardt et al., 2015). Phenolic and polyphenolic compounds are good indicators of honey botanical and geographical origin (Andrade, Ferreres, & Amaral, 1997; Stephens et al., 2010; Tomás-Barberán, Martos, Ferreres, Radovic, & Anklam, 2001; Yao et al., 2003). In addition to the characterisation and classification of honeys, fluorescence spectroscopy could also potentially detect unique intrinsic fluorophores and their relative concentrations, and inform on the physico-chemical parameters of the honey matrix (Lenhardt et al., 2015).

The distinctive phenolic and polyphenolic composition in New Zealand honeys, coupled to the high sensitivity of fluorescence spectroscopy, might therefore be expected to generate unique excitation–emission (ex–em) spectra identifiable to the individual honey types. Dilution of a honey by other honey floral types may result in a proportional change in the chemical composition of the honey and thus the fluorescence signal. We hypothesised that fluorescence arising from the unique chemical composition of New Zealand manuka and kanuka honeys might have utility to determine the relative floral contributions and therefore monoflorality.

The aim of this study was to examine the fluorescence characteristics of New Zealand honeys and establish a fluorescence screening method for estimating their monoflorality based on fluorescence profiles. Instead of generating full spectra for honeys, this study selectively identified unique ex–em wavelength pairs to screen a range of high-value New Zealand honeys. This selective approach greatly reduced the complexity of the scanning technique by isolating and targeting only characteristic ex–em wavelengths. Consequently, the analysis time was minimised making it more suitable for commercial applications that often involve screening a large number of samples.

Table 1
Representative New Zealand honeys, propolis, and nectar samples.

Sample	Honey/nectar	Geographic origin
1	Manuka (*Leptospermum scoparium*)	Northland
2	Manuka (*L. scoparium*)	Northland
3	Manuka (*L. scoparium*)	Northland
4	Manuka (*L. scoparium*)	Waikato Wetlands
5	Manuka (*L. scoparium*)	Waikato Wetlands
6	Manuka (*L. scoparium*)	East Coast
7	Manuka (*L. scoparium*)	East Coast
8	Manuka (*L. scoparium*)	Central North Island
9	Kanuka (*Kunzea ericoides*)	Northland
10	Kanuka (*K. ericoides*)	Northland
11	Kanuka (*K. ericoides*)	Northland
12	Kanuka (*K. ericoides*)	Northland
13	Kanuka (*K. ericoides*)	Waikato Wetlands
14	Rewarewa (*Knightia excelsa*)	Bay of Plenty, North Island
15	Rewarewa (*K. excelsa*)	North Island
16	Kamahi (*Weinmannia racemosa*)	South Island
17	Kamahi (*W. racemosa*)	North Island
18	Clover (*Trifolium* spp.)	South Island
19	Clover (*Trifolium* spp.)	South Island
20	Clover (*Trifolium* spp.)	North Island
21	Propolis (botanical source varies)	New Zealand
22	Nectar (*L. scoparium*)	Collected, Bay of Plenty, North Island
23	Nectar (*L. scoparium*)	Collected, Bay of Plenty, North Island
24	Nectar (*L. scoparium*)	Collected, Bay of Plenty, North Island
25	Nectar (*L. scoparium*)	Collected, Bay of Plenty, North Island
26	Nectar (*L. scoparium*)	Collected, Bay of Plenty, North Island