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An investigative study on discrimination of honey of various floral and geographical origins using UPLC-QToF MS and multivariate data analysis

Z. Jandrić*, R.D. Frew, L.N. Fernandez-Cedi, A. Cannavan

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 International Centre PO Box 100, Vienna, Austria

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ABSTRACT

An untargeted metabolomics approach was applied for the discrimination of honey of various floral and geographical origins using ultra-performance liquid chromatography-quadrupole time of flight mass spectrometry (UPLC-QToF MS). Evaluation of data by multivariate data analysis allowed the discrimination of New Zealand and Australian honeys from honeys originating in other countries. It was also possible to differentiate between mono- and polyfloral honeys within one region, as well as between some monofloral honeys of various geographical origins. Characteristic markers, contributing to the segregation of different honey types, were selected and characterized.

1. Introduction

The health benefits of honey have long been recognized. Some honey types (specific botanical and/or geographical origin) have enhanced medicinal and nutritional properties (Gannabathula et al., 2012; Kwakman et al., 2010; Tonks et al., 2007) and thus command a premium price over other honey types. Therefore, honey is one of the most common food commodities to be subject to mislabelling and fraud, and this has become an issue of international scale in terms of authenticity and quality control (Moore, Spink, & Lipp, 2012). A survey of U.S. honey packers reported that 71% of the firms that tested for economic adulteration in their honey supplies had found adulterated honey (Fairchild, Nichols, & Capps, 2003). The use of chloramphenicol, an antibiotic prohibited for use in food production in many countries, on bees in China resulted in a 2-year ban of Chinese honey in the EU and Canada (http://www.beeculture.com). There have been widespread reports that Chinese honey has been shipped to other countries, repackaged, and re-exported to avoid taxes and inspections (Ayres, 2008; Leeder, 2011). During the two-year EU ban, the disappearance of legal Chinese honey caused upheaval. For years it had been a basic ingredient in blended honeys because of its sweetness and cheapness; when it became unavailable packers worldwide switched to Argentinian, Mexican and east-European honeys, leading to similar charges of country-of origin labelling (http://www.theguardian.com). Recently, various instances of so-called 'honey laundering' on a large-scale have emerged, where honey was filtered to remove pollen or soil that could be used to trace it back to its origin (Leeder, 2011). Therefore, there is an ongoing need to explore other techniques that can complement and improve upon existing methods for tracing the origins of honey (Jandrić et al., 2015).

The majority of the honeys on the market contain significant nectar or honeydew contributions from several plant species and are therefore called polyfloral honeys. Usually they are labelled only with the word 'honey'. Honey may be designated according to floral or plant source if it comes wholly or mainly from that particular source and has the organoleptic, physicochemical and microscopic properties corresponding with that origin. Furthermore, where honey has been designated according to floral, plant source, or by the name of a geographical or topological region, then the name of the country where the honey has been produced shall be declared (Codex Alimentarius, 2001). Honey composition and properties depend not only on floral sources, but also on factors such as bee species, geographical area, production season, mode of storage, and harvest technology and conditions. Therefore, classification based

* Corresponding author.
E-mail address: z.jandric@iaea.org (Z. Jandrić).

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on floral and geographical origin can be very difficult and currently depends on a range of analyses (Persano Oddo, Piazza, Sabatini, & Accorti, 1995). Several methods have been published that are capable of characterizing specific monofloral honey types using targeted chemical classes and compounds as markers (Cavazza, Corradini, Musci, & Salvadeo, 2012; Chan et al., 2013; Kečkeš et al., 2013). The applications of those methods that are available are limited to a small number of specific honey types. To our knowledge, there is currently no untargeted method available for differentiating a variety of monofloral honeys on an international scale, as well as differentiating between mono- and polyfloral honeys within one region.

The aim of this study was to explore the feasibility of applying ultra-performance liquid chromatography-quadrupole time of flight mass spectrometry (UPLC-QToF MS) with multivariate statistical analysis (MVA) for the classification of honey of various botanical and geographical origins.

2. Materials and methods

2.1. Chemicals and reagents

Water and methanol (LC-MS grade) were supplied by Sigma–Aldrich (St. Luis, MO, USA). Formic acid was obtained from Thermo Scientific (Bellefonte, PA, USA).

2.2. Instrumentation

Chromatographic experiments were performed on a Waters ACQUITY™ UPLC™ system connected to Xevo G2 Q-ToF MS equipped with an electrospray ionization source (Waters, Milford, MA, USA). UPLC separation was achieved using an ACQUITY UPLC™ BEH C_{18} columns (100 × 2.1 mm, 1.7 μm) column. The mobile phases were 0.1% formic acid in water (A) and methanol (B), with a linear gradient elution: 0–0.75 min, A: 99%; 0.75–2.0 min, A: 99%–95%; 2.0–3.0 min, A: 95%; 3.0–6.5 min, A: 95%–45%; 6.5–8.5 min, A: 45%–10%; 8.5–9.0 min, A: 10%; 9.0–9.1 min, A: 10%–99%; 9.1–10 min, A: 99%. The flow rate of the mobile phase was 0.4 mL/min, and the column temperature was maintained at 45 °C. The injection volume was 3 μL. The gradient elution and mass spectrometric parameters were as described by Jandrić et al. (2014).

2.3. Sample preparation and analysis

Honey samples (n = 49) were collected from markets in 22 countries world-wide. For the purposes of this pilot study the samples were assumed to be authentic with regard to the country and floral origins on the labels (Table 1). The honey samples (0.5 g) were diluted with 10 mL of 1% formic acid in methanol/water (1:1, v/v), shaken (20 min), sonicated (20 min), and filtered through 0.2 μm PTFE membrane filter before injection. In parallel, quality control

Table 1
Honey samples obtained from retail markets.

Label on the package	Country of origin	Code	Floral type information	Number of samples
Attiki Greek honey	Greece	GRE	Thyme, herbs and wild flowers	1
Natural of Thassos	Greece	GRE	Unknown	1
Hnz manuka active UMF 10+	New Zealand	NZL	Manuka	1
Hnz manuka active UMF 6+	New Zealand	NZL	Manuka	1
Airborne kamahi	New Zealand	NZL	Kamahi	3
Choice Tasmanian meadow honey	Australia	AUS	Juicy clover pastures, brambles and wild flowers	1
Genuine Tasmanian leatherwood honey	Australia	AUS	Leatherwood	1
Le mas abeilles miel de Lavande	France	FRA	Lavender	1
Miel de Midi-Pyrenees fleur des terres de Gascogne	France	FRA	Gascony flowers	1
Le pere Gatounet miel de Provence	France	FRA	Provence flowers	1
Recolte artisanale Miel l'Apiculteur Des Alpes	France	FRA	Flowers, unknown	1
Piana miele millefiori apicoltura	Italy	ITA	Flowers, unknown	1
Darbo Naturrein feiner Akaziehonig	Austria	AUT	Acacia	1
Darbo NaturreinSonnenblumenhonig mit Sommertracht	Austria	AUT	Sunflower	1
Bergland Honig echter Waldbluetenhonig	Austria	AUT	Mix of flowers from meadow and woodland	1
Miel de Abeja Apis mellifera La Abeja Dorada	Columbia	COL	Unknown	1
Lapataia miel	Uruguay	URY	Unknown	1
Nzoke pure honeyasali ya nyuki wakubwa	Tanzania	TZA	The Miombo woodlands of Tabora	1
Flydende Blomsterhonning Dansk Honning	Denmark	DNK	Floating flowers	1
Elmehdi	Tunisia	TUN	Unknown	1
Wilkin & Sons LTD Exmoor blossom	United Kingdom	GBR	Moor plants	1
Wilkin & Sons LTD English borage	United Kingdom	GBR	Borage	1
Wilkin & Sons LTD English blossom	United Kingdom	GBR	Unknown	1
Fer Yayla Bali	Turkey	TUR	Wildflowers	1
Lokman suezme Cicek Bali	Turkey	TUR	Flowers, unknown	1
Koska suezme Cam Bali	Turkey	TUR	Pine	1
Sidr honey Hadrami	Saudi Arabia	SAU	Sidr	1
Cvjetni med	Macedonia	MKD	Flowers, unknown	1
Sumski med	Macedonia	MKD	Flowers, woodland	1
Granja San Francisco	Spain	ESP	Flowers, unknown	1
Miel de Gratallops priorat Mil Flors	Spain	ESP	Flowers, unknown	1
Miel de Gratallops priorat Farigola	Spain	ESP	Thyme	1
Eroski Mil Flores Dosificador Antigoteo	Spain	ESP	Flowers, unknown	1
Krnjevac cvetni med	Montenegro	MNE	Flowers, unknown	2
Dalmamed med cvjetni	Croatia	CRO	Flowers, unknown	1
Medino vranci livadski med	Serbia	SRB	Flowers, unknown	2
Bagremov med	Serbia	SRB	Acacia	1
Med livada	Bosnian	BIH	Flowers, unknown	3
Med vrijesak	Bosnian	BIH	Winter savory	1
Med zalfija	Bosnian	BIH	Sage	1
Wild honey Stung Treng	Cambodia	CAM	Wild honey	1
Wild honey Ratanakiri	Cambodia	CAM	Wild honey	1
Hero honey liquid Swiss jam tradition	Switzerland	SUI	Flowers, unknown	1

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