See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/352865950

The super-food Manuka honey, a comprehensive review of its analysis and authenticity approaches

Article *in* Journal of Food Science and Technology -Mysore- · June 2021 D0I:10.1007/s13197-021-05181-7

citations 0 reads 27

3 authors, including:

The American University in Cairo
289 PUBLICATIONS
8,907 CITATIONS

SEE PROFILE

Mohamed A Farag

Some of the authors of this publication are also working on these related projects:

Project Special issue on Plant Genomics View project

corals metabolomics View project

REVIEW ARTICLE



The super-food Manuka honey, a comprehensive review of its analysis and authenticity approaches

Nesrine M. Hegazi¹ · Ghada E. Abd Elghani² · Mohamed A. Farag^{3,4}

Revised: 30 May 2021 / Accepted: 15 June 2021 © Association of Food Scientists & Technologists (India) 2021

Abstract Manuka honey (MH) stands out from other honey types as a unique super-food with clinically proven antimicrobial and wound healing activities. Its unique traits and the broad range of applications (i.e. food, cosmetics, nutraceuticals /natural health products) have marked up its price 6 to 25 times than other honey types. Concurrent to the increased market demand, more fraudulence of MH emerged. This urged for the employment of analytical tools for the authenticity and quality assessment of MH and has been the focus of many researchers during the last decades. Our main focus was to review the literature dealing with MH authenticity during the period from 2010 to mid-2021 comprehensively via the Scifinder (https://sinfinder.cas. org) and Web of Science (https://webofknowledge.com) research engines. We used "manuka honey analysis", "manuka honey quality control", and "manuka honey authenticity" as a search terms, applied Boolean operators 'AND/OR' combination, performing in Jan 2017 from the following electronic databases. The state-of-the-art

Nesrine M. Hegazi nmhegazi@nrc.sci.eg; nesrinehegazi@yahoo.com

- Mohamed A. Farag Mohamed.farag@pharma.cu.edu.eg; Mohamed.alifarag@aucegypt.edu
- ¹ Phytochemistry and Plant Systematics Department, Division of Pharmaceutical Industries, National Research Centre, Cairo 12622, Egypt
- ² Chemistry Department, Faculty of Science, Mansoura University, Mansoura 35516, Egypt
- ³ Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt
- ⁴ Chemistry Department, School of Sciences & Engineering, The American University in Cairo, New Cairo, Egypt

analytical approaches and respective chemical markers of MH are highlighted. The present study capitalizes on the most updated methodologies employed for the quality control and analysis of MH to ensure its authenticity and adulteration detection. The unique constituents of MH allowed for its successful discrimination through various analytical platforms, including mass spectrometry coupled to suitable chromatographic separation (i.e. GC–MS and LC–MS), nuclear magnetic resonance (NMR), and fluorescence analysis. Moreover, chemometric tools present potential for MH discrimination and has yet to be capitalized more upon for MH quality control analysis.

Keywords Authenticity · Chemical markers · Manuka honey · Methylglyoxal · Leptoserin and leptridine

Abbreviations

MH	Manuka Honey	
QC	Quality control	
UMF	Unique Manuka Factor	
GC-MS	Gas Chromatography-Mass Spectrometry	
HPLC	High-Performance Liquid Chromatography	
NMR	Nuclear Magnetic Resonance	
ED-XRF	Energy-Dispersive X-Ray Fluorescence	

Introduction

Since ancient times, honey has been acknowledged for its many health benefits besides being used as a sweetener and in cosmetics. Recently, it has been the focus of many research projects to re-explore its unique properties. Of the different types of honey, Manuka honey (MH) has gained much popularity worldwide owing to its substantial antibacterial properties. MH is produced exclusively in New Zealand and some parts of Australia being the habitat of *Leptospermum scoparium* (Manuka tree). The nectar of the Manuka tree is exceptionally rich in dihydroacteone (DHA), the precursor of methylglyoxal (MGO). MGO is formed during MH honey maturation and aging and is primarily accountable for its distinct antibacterial properties (Adams et al. 2009), and several other health benefits. These unique properties increased the demand for the scarce MH and heightened its vulnerability to fraud.

According to the guardian, the economic importance of MH has recently heightened, with the values of its export increasing by fivefold in 2018 giving rise to the manuka "gold rush". This escalating demand led to increased adulteration of MH with claims that half of the marketed MH is not genuine (The Guardian 2018). MH fraud may occur in many ways to include the addition of sugar syrups and mislabeling the geographical and botanical origin (Wang et al. 2021).

Traditionally, physicochemical properties were employed for the analysis and quality assessment of honey (Dong et al. 2016, Kavanagh et al. 2019). Nevertheless, conventional analytical tools show limitations in adulteration detection of MH owing to its complexity as a typical food matrix. Recently, high-performance liquid chromatography (Yao et al. 2003, Locher et al. 2017), and nuclear magnetic resonance spectroscopy (Spiteri et al. 2017) were also employed for the analysis of honey. In this regard, this review shall cover the state-of-the-art analytical tools for the adulteration detection and quality control assessment of MH highlighting the applications, advantages, and limitations of each technology.

MH quality control analysis

Owing to its ample antibacterial properties, there is an escalating commercial demand for MH which may increase its liability for adulteration. Traditionally, honey authentication relied mostly on pollen grains analysis, however, this approach proved irrelevant for MH verification as the Manuka plant shares identical pollens with the concurrent flowering Kanuka bush (Spiteri et al. 2017).

Whereas, the physicochemical analysis of honey mainly detects sugar adulteration with increased amounts of sucrose or maltotriose of distinct composition from natural honey sugars. However, sugars with similar isotope fingerprint to honey (*i.e.* rice, beet, etc.) though are not observable using these methods (Bogdanov et al. 2004). Moreover, it provides little information about its botanical and/ or geographical origin.

Overall, MH quality control assessment can follow the same protocol as any other honey type, though with some precautions and/or needed modifications. For example, the high levels of MGO in MH may interfere with sugar analysis (AOAC 998.12). Likewise, the C4 sugar detection method (AOAC 2013) attributed to the interaction of MH active ingredients with honey proteins resulting in large isotope differences that can offset C4 sugar calculations (Rogers et al. 2014). High levels of DHA and/or MGO in MH, react with honey proteins causing isotopic fractionation thus enhancing the apparent C-4 sugar value (Rogers et al. 2014).

MH is valued for its phenomenal antibacterial properties which is mostly attributed to its high content of MGO (Beitlich et al. 2014). MH is marketed commercially by its UMF (Unique Manuka Factor) which was established to specify its exclusive MGO content (Girma et al. 2019).

Recently, the analysis of secondary plant metabolites (volatile and nonvolatile constituents) present in MH showed potential for its discrimination from other uni-floral honeys and its quality assurance. Following such hypothesis, the New Zealand Ministry for Primary Industries, NZ-MPI launched a science program in 2013 (MPI 2013) to define the fine characteristics of MH which were developed over time (MPI 2014) to the final NZ-MPI for the powerful and reliable testing of MH (MPI 2017a, 2017b, 2017c). The MPI-MH definition relies on the analysis of few, albeit specific markers of MH via simple and sensitive analytical approaches based on four chemical attributes (3-phenyllactic acid, 2'-methoxyacetophenone, 2- methoxybenzoic acid, and 4-hydroxyphenyllacetic acid) (MPI 2017b) and DNA pollen analysis by quantitative polymerase chain reaction (qPCR) (MPI 2017c).

Factors for determining the authenticity of MH were previously reviewed by (Burns et al. 2018). Consequently, and in the following sections, expansion on the state-ofthe-art analytical platforms used for MH analysis (**Table 1**) shall be discussed with highlights on each technique's advantages and/or limitations.

Chromatographic techniques

Several studies targeted quantification of MGO, the characteristic antibacterial component of MH using Reverse Phase (RP)-HPLC coupled with UV detection (Pappalardo et al. 2016). Nevertheless, analysis of MGO or its precursor dihydroxyacetone has shown limitations due to the commercial availability of these ingredients which could be added to upgrade other honeys with lower antibacterial activities (Beitlich et al. 2016). Comparably, the analysis of specific markers or residual enzymatic activities was of restricted application to some cases (Xue et al. 2013).

Comparative profiling of uni-floral honeys' phenolic profile (*i.e. Leptospermum* honeys) from different geographical origins (*i.e.* Australian vs. New Zealand) and/or

Methodology	Application	Results	References
SPME -GC/MS	Assessment of volatile profile of MH versus Kanuka honey	 2-methylbenzofuran, 2'-hydroxyacetophenone, and 2'- methoxyacetophenone are characteristic for MH <i>versus</i> 2,6,6-trimethyl-2-cyclohexene-1,4-dione, phenethyl alcohol, <i>p</i>-anisaldehyde for Kanuka honey 	Beitlich et al. (2014)
HPLC	Comparative profiling of unifloral honeys' phenolic profile	Chrysin was found characteristic for MH <i>versus</i> myricetin in jelly bush honey (<i>L. polygalifolium</i>), with enrichment of gallic and abscisic acid in MH	Yao et al. (2003)
LC-MS	Qualitative determination of MH antioxidant metabolites	MH showed an apparent capability of scavenging superoxide anions as revealed using LC-selective for the analysis of electroactive compounds and to identify methyl syringate as a major antioxidant	Inoue et al. (2005)
HPLC-MS	Analysis of MH and other uni-floral honeys	Qualitative variation among honeys associated with the floral origin, geographical region, and storage conditions	Fearnley et al. (2012)
UPLC-PDA-MS/ MS	Determination of specific chemical markers for MH authentication	Improved separation power and a higher sensitivity level for the detection of minor MH components	Beitlich et al. (2014)
Fluorescence Spectroscopy	MH authentication	Rapid, simple, and high throughput on-site honey analyses	(Bong et al. 2016, Lin et al. 2020), Lin et al. (2020), Bong et al. (2017)
NMR spectroscopy coupled with chemometrics	-Detection of adulterated MH	-Identification of respective markers from sugar syrups i.e., glucose, fructose, sucrose, and 5-hydroxymethyl furfural	Spiteri et al. (2017)
	-Differentiation of honeys of different botanical origins	-Identification of MGO, DHA, and leptosperin as unique markers of MH	

Table 1 Different analytical methodologies employed for the analyses and authentication of MH

SPME–GC-MS: Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry; LC–MS: Liquid Chromatography-Mass Spectrometry; HPLC–MS: High-Performance Liquid Chromatography-Mass Spectrometr—UPLC-PDA-MS/MS: Ultra Performance Liquid Chromatography-Photodiode Array—Tandem Mass Spectrometry; NMR: Nuclear Magnetic Resonance

genotype (*i.e. L. scoparium vs. L. polygalifolium*) was performed using HPLC. Qualitative and quantitative differences in flavonoid and phenolic acid profiles were observed between both honey types. For instance, chrysin was found characteristic for MH *versus* myricetin in jelly bush honey (*L. polygalifolium*), with enrichment of gallic and abscisic acid in MH. These observed species differences advocate the possible authentication of MH through the analysis of its phenolic profile (Yao et al. 2003), and suggestive for species-specific markers among these two *Leptospermum* honeys. These results also confirm that HPLC analysis can be an objective tool for honey floral authentication targeting its non-volatile metabolome.

While, qualitative determination of MH antioxidant principles was performed using liquid chromatography coupled to colorimetric array detection, and mass spectrometry (LC-MS), absolute quantification using these methods though has yet to be validated. Compared to other uni-floral honeys, MH showed an apparent capability of scavenging superoxide anions as revealed using LC-selective for the analysis of electro-active compounds and to identify methyl syringate as a major antioxidant (Inoue et al. 2005).

Additionally, the concurrent quantification of leptosperin and lepteridine via high- HPLC-UV- MS or fluorescence spectroscopy was proposed for the reliable authentication of MH (Lin et al. 2020) (Fig. 1, Yellow, grey and green colors correspond to volatile, nonvolatile and flourscent markers in MH, respectively), with quite similar results to the regulatory definition by the New Zealand authorities which advises the co-employment of metabolic fingerprinting with pollen DNA polymerase chain reaction (PCR) (Burns et al. 2018). Considering that other MH constituents which contribute to its antibacterial properties, an analytical approach was proposed for the analysis of MH antibacterial compounds (i.e. MGO, DHA, H₂O₂, fructose, and glucose). A simple and rapid analysis of the reactive oxygen species (ROS) produced by MGO, DHA, fructose, and glucose together with the existing H_2O_2 was described using a lucigenin chemiluminescence method (lucigenin-CL) coupled to HPLC (Karasawa et al. 2017). Furthermore, the O₂- and H₂O₂- derived luminescence was differentiated pre- and post-treatment with catalase or superoxide dismutase to assess the contribution of ROS levels in MH (Karasawa et al. 2017).

Fig.1 Significant chemical markers of MH and the respective analystical technique for analysis. Yellow, grey and green colors correspond to volatile, nonvolatile and flourscent markers in MH, respectively



(Beitlich et al. 2014).

Mass spectrometry coupled to chromatographic seperation

Mass spectrometry coupled to Gas chromatography

Solid-phase micro-extraction (SPME) coupled to gas chromatography-mass spectrometry (GC–MS) was employed for the analysis of MH volatile constituents, and identified methyl syringate, 2'-methoxyacetophenone, and 3-phenyllactic acid as the main volatile markers in MH (**Fig. 1**) (Daher and Gulacar 2010), while furfurals (*i.e.* 2-furfural & 5-methyl-2-furfural) were indicators of MH quality and freshness indices (Gras et al. 2014).

The volatile profile of MH *vs* its closely related Kanuka honey was analyzed via headspace-solid phase micro-extraction (HS-SPME)-GC/MS coupled to chemometrics. MH is distinctive with 2-methylbenzofuran, 2'-hydroxyacetophenone, and 2'-methoxyacetophenone. Whereas 2,6,6-trimethyl-2-cyclohexene-1,4-dione, phenethyl

Mass spectrometry coupled to liquid chromatography

alcohol, *p*-anisaldehyde are distinctive to Kanuka honey

Whereas GC is more suited for volatile analysis of MH to account for its sensory characters or freshness indices, HPLC provides a better readout of secondary bioactive components mediating for its health benefits.

Isotope ratio mass spectrometry coupled to liquid chromatography (LC-IRMS) was proposed to detect the originality of MH through the determination of δ^{13} C values of honey corresponding to the extracted protein and single sugars (i.e. sucrose, fructose, and glucose) favorable evidence in authenticity identification of honey with a C-4 sugar content of < 0%. However, it showed limitations when sugar syrups with honey-like isotopic patterns were added (Dong et al. 2016). Additionally, the analysis of complex multi-component samples through LC/IRMS is quite difficult which could be overcome by the use of twodimensional (2D)-LC (Suto et al. 2019) offering improved peak capacity, selectivity, and resolution. The analysis of organic acids in MH post-separation from carbohydrates was performed via IRMS. 2D-LC proved more effective in isotopic research and is suggested to find application in analyzing complex samples (Suto et al. 2019).

Fourier-transform mass spectrometry coupled to HPLC was employed for MH profiling in the context of its different geographical regions in New Zealand, different ages $(\leq 1-5 \text{ yrs})$, and storage conditions (4 °C vs. room temperature) versus other uni-floral honey (i.e. clover honey). A wide array of low molecular weights were detected showing qualitative variation with the floral origin, geographical region, and storage conditions. Further, a large divergence among the detected compounds was seen between Manuka and clover honeys. MH stored at room temperature showed a higher number of peaks revealing storage impact on honey composition which could be, attributed to the breakdown of high molecular weight compounds (e.g. polymers and waxes) (Fearnley et al. 2012). Conclusively, further studies should be conducted to unravel the underlying changes in honey composition during storage and how it affects its biological activities.

Another impact of storage conditions and heat treatment in honey lies in the formation of high molecular weight melanoidins polymers produced by *Maillard* reaction (Borrelli et al. 2002). Melanoidins are formed during heat treatment of food and confer to their sensory traits (*i.e.* color, texture, and taste). LC–ESI–MS coupled to size exclusion chromatography (SEC) of MH revealed that thermal processing led to the incorporation of polyphenols into melanoidin fraction forming complexes and to interestingly further enhance melanoidins' antioxidant properties (Brudzynski and Miotto 2011).

The determination of specific chemical markers for MH authentication is pivotal for its QC assessment. For this purpose, comprehensive ultra-performance liquid chromatography-photodiode array (UPLC-PDA)-MS/MS analysis was employed for the discrimination of MH from its closely related mono-floral honey (i.e. Kanuka and jelly bush honey) followed by multivariate analysis. Compared to HPLC, UPLC provides increased separation power and a higher sensitivity level for the detection of minor MH acetyl-2-hydroxy-4-(2-methoxcomponents suc as yphenyl)-4-oxobutanate (Beitlich et al. 2014). The distinctive component of MH was identified as leptosperin (Fig. 1), versus 4-methoxyphenyllactic acid and methyl syringate in Kanuka honey (Beitlich et al. 2014), and in agreement with Kato et al. (2012). Multivariate analysis did not though allow for a clear classification as these markers were not specific for only one honey type (Beitlich et al. 2014).

Furthermore, proteomics is recently employed for food authentication and has been optimized for the qualitative analysis of MH. The employed bottom-up proteomic approach based on nanoLC-QqTOF-MS/MS allowed for the identification of 50 bee-derived proteins with the dominance of the major royal jelly proteins (MRJPs). Among the identified peptides, 12 were found unique to MH endorsing the potential use of peptide profiling for MH authentication (Bong et al. 2021).

Equally important in MH quality assessment is the detection of pesticides that not only affect its quality but rather safety. The analysis of residual fenpyroximate pesticide in different honey samples to include MH was established via LC–MS/MS analysis post-liquid–liquid extraction and solid-phase extraction to remove possible interferences from sugars and proteins in the complex honey matrix, with a detection limit of 0.05 μ g kg⁻¹ (Kim and Myung 2018).

Lastly, the concurrent use of both HS-SPME-GC/MS and UHPLC-PDA-MS/MS combined with statistics (HAHSUS protocol: honey authentication by HS-SPME-GC/MS and UHPLC-PDA-MS/MS) was recommended for the verification of honey origin, and for the quantitative estimation of MH in mixed honeys (Beitlich et al. 2016).

Fluorescence spectroscopy

Recently, fluorescence spectroscopy has been validated for the quality control and analysis of a wide range of food to include dairy products, oils, and honeys (Lin et al. 2017). Fluorescence spectroscopy demonstrated higher sensitivity than other spectroscopic tools with a quantification limit reaching 1 ppb (Karoui 2018) in addition to being simple, reliable, and economic. Fluorescence properties of honey may be attributed to its phenolic constituents, amino acids, and/ or products of Maillard reaction (Stephens et al. 2017).

Fluorescence spectroscopy showed potential for MH authentication via the identification of exclusive fluorescence markers namely MM1 (ex270–em365 nm) and MM2 (ex330–em470 nm) (Bong et al. 2016) which were later identified as leptoserin (phenolic acid glycoside) (Bong et al. 2017) and lepteridine (pteridine derivative) (Daniels et al. 2016) (Lin et al. 2017, Bong et al. 2018) (Fig. 1), respectively. The stability of lepteridine over time can be advantageous for the rapid, simple, and high throughput on-site honey analyses using portable fluorescent devices (Lin et al. 2020).

Similarly, fluorescence properties of MH proteins versus non-MH were characterized to highlight the glycation reaction between carbonyl compounds (*i.e.* MGO) and MH proteins. An increase in fluorescence intensity was observed and correlated with MGO levels and storage conditions. The increased fluorescence resulted from protein adducts produced via MGO derived reactions versus the formation of disulfide bonds in non-MHs. These specific fluorescences at λ_{ex} 330 and λ_{em} 470 nm in MH could be used for the authentication of genuine MH as they were not observed when non-MHs were spiked with MGO or DHA (Rueckriemen et al. 2017).

Nuclear magnetic resonance spectroscopy

The use of 1D-nuclear magnetic resonance (NMR) and diffusion ordered spectroscopy (DOSY-NMR) for the quantitative and qualitative analysis of complex mixtures has been well reported in several food matrices (Consonni and Cagliani 2008, Adams et al. 2011). NMR offers the advantage of being non-destructive and requires minimal sample pretreatment offering rapid, reliable, and efficient tools for analysis. Additionally, several commercial databases are available for food authentication (i.e. fruit juices, wine, and honey). For instance, the Bruker database includes more than 8000 references for honey adulteration (Kuballa et al. 2018).

In that context, the feasibility of using DOSY-NMR for the assessment of MH components concerning their antibacterial activity was reported (Le Gresley et al. 2012). The adopted methodology allowed for the qualitative and quantitative detection of aliphatic (*i.e.* methylglyoxal dehydrate, methylglyoxal monohydrate, lactic acid, pyruvaldehyde, and pyruvic acid) as well as some aromatic (*i.e.* phenyllactic acid, 3,4,5-trimethoxybenzoic acid, and methyl syringate) components of MH.

The development of ¹H-NMR methodology to detect adulteration in MH allowed for the identification of respective markers from sugar syrups i.e., glucose, fructose, sucrose, and 5-hydroxymethyl furfural. Additionally, NMR signals indicative of sugar degradation or fermentation (*i.e.* lactic acid, succinic acid, acetic acid, and ethanol formation) were observed. The differentiation of MH from other mono-floral honeys using MH specific markers (*i.e.* MGO & DHA) was successful based on their NMR spectra. DHA is a marker of MH quality and freshness, being present at the highest levels in freshly harvested MH and decreasing proportionally during its ripening (Spiteri et al. 2017).

Honey samples from the Oceania region (New Zealand & Australia) were examined by ¹H-NMR coupled with chemometrics, with the identification of MGO, DHA, and leptosperin as unique markers of MH found reliable for the assessment of honey botanical origin (Spiteri et al. 2017). Moreover, the accuracy of multivariate data analysis in sample discrimination, *i.e.* partial least squares discriminant analysis (PLS-DA) was exploited with a model constructed to differentiate MH from other mono-floral honeys

based on the NMR profile of the three mentioned markers (Spiteri et al. 2017).

DNA analysis

DNA markers were used for the verification of MH authenticity via quantitative pollen analysis polymerase chain reaction (qPCR) to detect the DNA of *L. scoparium* (McDonald et al. 2018). However, the practical application of this approach is rather limited due to its high cost.

Equivalently, plant miRNAs (microRNAs) are plantderived and present in honey. The composition of Australian honey and New Zealand MH RNA molecules were cataloged using next-generation sequencing (NGS). Results revealed the presence of small non-coding RNAs which could be derived from the plant, invertebrates, and/or the prokaryotic species. Additionally, it revealed the existence of unique small RNA profiles which could be further exploited for honey authentication (Smith et al. 2021).

Other techniques

Aquaphotomics is a new "omics" discipline aiming to detect changes in the water molecular system in biological and aqueous systems by observing the water electromagnetic (EM) spectrum of those systems under various perturbations (van de Kraats et al. 2019). Aquaphotomics relies on the slight changes in water molecular system arising from near-infra-red (NIR) spectroscopy, and to provide a rapid, low-cost method for detecting changes in water molecular arrangement as a biomarker of water quality (Kovacs et al. 2016). Water structure in MH contributes to its biological properties. Syrup addition to MH may alter these functional water structures. The feasibility of detecting MH adulteration with different sugar syrups (corn syrup, sucrose syrup, high fructose corn syrup, beet syrup, and rice syrup) using aquaphotomics based NIR spectroscopy. 12 Characteristic bands in the spectral region of 1300-1800 nm were chosen for the aquagrams construction. The aquagram revealed the more structured water in MH versus more free water molecules in sugar syrups. The proposed protocol comprising NIR spectroscopy coupled with aquaphotomics granted a fast and simple method for the detection of syrup addition to MH (Yang et al. 2020).

Additionally, an immunochromatographic test for MH authentication using a leptosperin-specific monoclonal antibody was developed and showed comparable results to those obtained by high-performance liquid chromatography (HPLC) or enzyme-linked immunosorbent assay (ELISA) (Kato et al. 2016).

Conclusion

This comprehensive review shed the light on the unique chemical constituents and quality control assessment of MH. MH's distinct chemical makeup includes flavonoids, phenolic acids, 1,2-dicarbonyl compounds, norisoprenoids, and volatile aromatic compounds. The commercial value and quality of MH is mainly viewed by its MGO content and is expressed through the UMF. Nevertheless, the UMF presents limitations in the determination of MH authenticity, as it gives no information on other constituents warranting for other analytical technologies to assess its quality and its discrimination from other uni-floral honeys. This review presents the different analytical techniques highlighting each advantages and/or limitations. SPME-GC-MS/MS analysis targeting MH aroma profile revealed 2-methylbenzofuran, 2'-hydroxyacetophenone, and 2'methoxyacetophenone as distinctive markers. While analysis of its non-volatile metabolites using HPLC-MS/MS analysis proved potential for its authentication via its unique glycoside leptosperin. Additionally, flourscenent markers (leptoserin and leptridine) proved the potential for robust and reliable authentication of MH). Similarly, a direct NMR metabolomics approach was found effective for MH adulteration detection (*i.e.* sugar syrup addition), differentiation of MH from other mono-floral honeys, and freshness indices determination. The application of multivariate analysis and, in particular, PCA, PLS proved to be extremely useful for grouping and detecting honeys of various origins and has yet to be fully applied in MH. Moreover, the inclusion of bioassays that can determine enzyme activities or biological effects in MH in parallel to chemical analysis should be now be pursued.

To conclude, MH authentication warrants the employment of a systematic interdisciplinary approach exploiting both chemical and DNA markers, in conjunction with appropriate data analysis for improved QC protocols of MH. Development of biosensors for QC management of MH seems more suited for the onsite analysis and for quick judgment of MH specimens.

Authors' contributions NMH, Literature survey, collection and writing the MS. GEA, Literature survey and collection. MAF, Review design and conceptualization, revising and editing the MS.

Funding Dr M. A. Farag thanks the Alexander von Humboldtfoundation, Germany, for financial support.

Declarations

Conflict of interest The authors declare no conflict of interests.

References

- Adams CJ et al (2009) The origin of methylglyoxal in New Zealand manuka (*Leptospermum scoparium*) honey. Carbohyd Res 344(8):1050–1053
- Adams RW et al (2011) Resolving natural productepimer spectra by matrix-assisted DOSY. Org Biomol Chem 9(20):7062–7064
- AOAC (2013). AOAC Official Method 998.12, C-4 plant sugars in honey, first revision. AOAC International, Gaithersburg, MD, USA: 44.44.18A
- Beitlich N et al (2014) Differentiation of manuka honey from kanuka honey and from jelly bush honey using HS-SPME-GC/MS and UHPLC-PDA-MS/MS. J Agric Food Chem 62(27):6435–6444
- Beitlich N et al (2016a) Fluorescent pteridine derivatives as new markers for the characterization of genuine monofloral New Zealand manuka (*Leptospermum scoparium*) honey. J Agric Food Chem 64(46):8886–8891
- Bogdanov S et al (2004) Physico-chemical methods for the characterisation of unifloral honeys: a review. Apidologie 35(Suppl. 1):S4–S17
- Bong J et al (2016) Fluorescence markers in some New Zealand honeys. Food Chem 192:1006–1014
- Bong J et al (2017) Leptosperin is a distinct and detectable fluorophore in *Leptospermum* honeys. Food Chem 214:102–109
- Bong J et al (2018) New approach: chemical and fluorescence profiling of NZ honeys. Food Chem 267:355–367
- Bong J et al (2021) Proteomic analysis of honey. Identification of unique peptide markers for authentication of NZ mānuka (*Leptospermum scoparium*) honey. Food Chem 350:128442
- Borrelli RC et al (2002) Chemical characterization and antioxidant properties of coffee melanoidins. J Agric Food Chem 50(22):6527–6533
- Brudzynski K, Miotto D (2011) Honey melanoidins: Analysis of the compositions of the high molecular weight melanoidins exhibiting radical-scavenging activity. Food Chem 127(3):1023–1030
- Burns DT et al (2018) A critical review of the factors available for the identification and determination of mānuka honey. Food Anal Methods 11(6):1561–1567
- Consonni R, Cagliani LR (2008) Geographical characterization of polyfloral and acacia honeys by nuclear magnetic resonance and chemometrics. J Agric Food Chem 56(16):6873–6880
- Daher S, Gulacar FO (2010) Identification of new aromatic compounds in the New Zealand manuka honey by gas chromatography-mass spectrometry. E-J Chem 7(Suppl. 1):S7–S14
- Daniels BJ et al (2016) Isolation, Structural Elucidation, and Synthesis of Lepteridine From Manuka (*Leptospermum scoparium*) Honey. J Agric Food Chem 64(24):5079–5084
- Dong H et al (2016) Adulteration identification of commercial honey with the C-4 sugar content of negative values by an elemental analyzer and liquid chromatography coupled to isotope ratio mass spectroscopy. J Agric Food Chem 64(16):3258–3265
- Fearnley L et al (2012) Compositional analysis of manuka honeys by high-resolution mass spectrometry: Identification of a manukaenriched archetypal molecule. Food Chem 132(2):948–953
- Girma A et al (2019) Antibacterial activity of varying UMF-graded Manuka honeys. PLoS One 14(10):e0224495
- Gras K et al (2014) Determination of furfurals in Manuka honey using piston-cylinder liquid-liquid extraction and gas chromatography. J Chromatogr A 1362:43–48
- The Guardian (2018) New Zealand brings first 'fake mānuka honey' prosecution
- Inoue K et al (2005) Identification of phenolic compound in manuka honey as specific superoxide anion radical scavenger using

electron spin resonance (ESR) and liquid chromatography with coulometric array detection. J Sci Food Agric 85(5):872–878

- Karasawa K et al (2017) Novel assay of antibacterial components in manuka honey using lucigenin-chemiluminescence-HPLC. Anal Chim Acta 954:151–158
- Karoui R (2018) Spectroscopic Technique: Fluorescence and Ultraviolet-Visible (UV-Vis) Spectroscopies. Elsevier, Modern techniques for food authentication, pp 219–252
- Kato Y et al (2012) Identification of a novel glycoside, leptosin, as a chemical marker of manuka honey. J Agric Food Chem 60(13):3418–3423
- Kato Y et al (2016) Competitive immunochromatographic assay for leptosperin as a plausible authentication marker of manuka honey. Food Chem 194:362–365
- Kavanagh S et al (2019) Physicochemical properties and phenolic content of honey from different floral origins and from rural versus urban landscapes. Food Chem 272:66–75
- Kim JM, Myung S-W (2018) Determination of Fenpyroximate from four types of honey by liquid chromatography/tandem mass spectrometry. Bull Korean Chem Soc 39(1):65–70
- Kovacs Z et al (2016) Water spectral pattern as holistic marker for water quality monitoring. Talanta 147:598–608
- Kuballa T, Brunner TS, Thongpanchang T, Walch SG, Lachenmeier DW (2018) Application of NMR for authentication of honey, beer and spices. Curr Opin Food Sci 19:57–62
- Le Gresley A et al (2012) The application of high resolution diffusion NMR to the analysis of manuka honey. Food Chem 135(4):2879–2886
- Lin B et al (2017) Lepteridine as a unique fluorescent marker for the authentication of manuka honey. Food Chem 225:175–180
- Lin B et al (2020) Utility of the *Leptospermum scoparium* Compound Lepteridine as a Chemical Marker for Manuka Honey Authenticity. ACS Omega 5(15):8858–8866
- Locher C et al (2017) Authentication of honeys of different floral origins via high-performance thin-layer chromatographic fingerprinting. JPC-J Planar Chromatography-Modern TLC 30(1):57–62
- McDonald CM et al (2018) Using chemical and DNA marker analysis to authenticate a high-value food, manuka honey. NPJ Sci Food 2:9
- MPI (2013). Options for defining monofloral manuka honey. MPI Discussion Paper No:2013/38. MPI New Zealand Government.
- MPI (2014). Science and characterising mānuka honey. Current and future science to support a definition. MPI Technical Paper No:2014/23. MPI, New Zealand Government.
- MPI (2017a). Criteria for identifying mānuka honey. MPI Technical Paper No: 2017/28. MPI, New Zealand Government.
- MPI (2017b). Determination of four chemical characterisation compounds in honey by liquid chromatography tandem mass

spectrometry (LCMS/MS). MPI Technical Paper No: 2017/30. MPI, New Zealand Government.

- MPI (2017c). Multiplex qPCR for detection of *Leptospermum* scoparium DNA from pollen in Honey. MPI Technical Paper No 2017/31. MPI New Zealand Government.
- Pappalardo M et al (2016) Rapid and reliable HPLC method for the simultaneous determination of dihydroxyacetone, methylglyoxal and 5-hydroxymethylfurfural in leptospermum honeys. PLoS One 11(11):e0167006
- Rogers KM et al (2014) The unique manuka effect: why New Zealand manuka honey fails the AOAC 998.12 C-4 sugar method. J Agric Food Chem 62(12):2615–2622
- Rueckriemen J et al (2017) Unique fluorescence and high-molecular weight characteristics of protein isolates from manuka honey (*Leptospermum scoparium*). Food Res. Int. 99(Part_1):469–475
- Smith C et al (2021) Cataloguing the small RNA content of honey using next generation sequencing. Food Chem: Molecular Sci 2:100014
- Spiteri M et al (2017) Combination of 1H NMR and chemometrics to discriminate manuka honey from other floral honey types from Oceania. Food Chem 217:766–772
- Stephens JM, Loomes KM, Braggins TJ, Bong J, Lin B, Prijic G (2017) Fluorescence: a novel method for determining manuka honey floral purity. Intech, pp 95–113
- Suto M et al (2019) Heart-cutting two-dimensional liquid chromatography combined with isotope ratio mass spectrometry for the determination of stable carbon isotope ratios of gluconic acid in honey. J Chromatography A. 1608:460421
- van de Kraats EB, Munćan J, Tsenkova RN (2019) Aquaphotomics– Origin, concept, applications and future perspectives. Substantia, pp 13–28
- Wang Z et al (2021) Recent advances in analytical techniques for the detection of adulteration and authenticity of bee products–A review. Food Additives & Contaminants: Part A 38(4):533–549
- Xue X et al (2013) 2-Acetylfuran-3-glucopyranoside as a novel marker for the detection of honey adulterated with rice syrup. J Agric Food Chem 61(31):7488–7493
- Yang X et al (2020) "Manuka honey adulteration detection based on near-infrared spectroscopy combined with aquaphotomics. LWT. 132:109837
- Yao L et al (2003) Flavonoids, phenolic acids and abscisic acid in Australian and New Zealand *Leptospermum* honeys. Food Chem 81(2):159–168

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.