Real-time assessment of Western Australian honey

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Foreword

Australian beekeepers and packers do not generally test their honey unless the testing is requested during the export process. The main reason for this is the test cost and with no price difference paid by Australian customers for this information, there is no incentive. The testing that is done for the export market is related to health issues, such as agriculture chemicals, antibiotics, and correct product identification as honey with no syrup adulteration. None of this testing is linked to the differentiation and marketing of the product, and this has been to the detriment of Australian honey appreciation and price.

This project addressed the cost of honey testing by determining how many honey tests can be done on a single machine and the relevance of these tests to the local market. Evident is that international tourists to Australia are a driver to developing export markets, so quality control of our local product will grow export opportunities.

Finding a simple analytical system to detect specific nectars that the honey bees have gathered to ripen into honey is a breakthrough for Australian honey. Many of our kinds of honey are monoflorals, that is that they have more than half the nectar from one flower source, as the bees are harvesting from deep-rooted ancient trees that can produce nectar flows that can support mammals and birds. Linking this nectar signature to honey offers a unique marketing tool that not only signifies a sense of place and season but also indicates an authentic product; with links being able to be made to the health attributes related to that honey source.

So, in finding a cheap honey testing alternative, using high-performance thin layer chromatography provided a unique and powerful marketing story unique to Australia.

Dr Liz Barbour
CEO

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About the Authors

Md Khairul Islam has a Master's and a Bachelor of Pharmacy from the University of Rajshahi in Bangladesh. He is currently a PhD student with the CRC for Honey Bee Products and the Division of Pharmacy, School of Allied Health at the University of Western Australia. Before commencing his PhD, candidature was a Lecturer at the Department of Pharmacy, Varenra University in Rajshahi, Bangladesh and worked as a Product Executive (Marketing) in the Product Management Department of Opsonin Pharmaceuticals in Bangladesh.

Dr Cornelia Locher (Staatsex. Pharm UniTü, PhD NTU) is an Associate Professor in Pharmaceutical and Medicinal Chemistry and Deputy Director of the Division of Pharmacy at the University of Western Australia. Her main research interests include the phytochemical analysis of medicinal honey, the isolation and identification of bioactive compounds, particularly from traditional Aboriginal and Asian medicinal plants, and pediatric formulations. Previously, Dr Locher was employed as Senior Lecturer in Pharmaceutical Chemistry at the School of Pharmacy, Curtin University (Perth, Australia), she also taught at the Northern Territory University (Darwin, Australia) and worked as a pharmacist in a community pharmacy in Germany.

Acknowledgments

We would like to thank the honey bee industry, particularly the Bee Industry Council of WA and the Tasmanian Beekeepers Association, for supplying the honey samples and review of our progress to develop this HPTLC system. The monofloral nectar identification we currently have completed is thanks to their commitment.

We would like to thank Tom Sostaric from Chromatech Scientific for his invaluable input and support throughout this project.

The collaboration and support from the Division of Pharmacy, Centre of Optimisation of Medicines School of Allied Health in the School of Allied Health at the University of Western Australia enabled an inspiring environment within which to work.

Abbreviations

DHA	Dihydroxyacetone
DPPH	2,2-Diphenyl-1-picryl-hydrazyl-hydrate
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic acid equivalent
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
IR	Infrared
MGO	Methylglyoxal
NMR	Nuclear Magnetic Resonance
TE	Trolox Equivalent
UV	Ultraviolet

Executive Summary

This project explores the use of High-Performance Thin Layer Chromatography (HPTLC), as a novel tool for the analysis of various aspects of honey chemistry. This methodology is used extensively in the natural products industry for authentication and quality control.

Methods have been developed for the authentication of a honey's nectar source via HPTLC fingerprinting of its organic extract coupled with multivariate data analysis; the detection and quantification of honey constituents that contribute to its antioxidant activity via a novel validated HPTLC-DPPH analysis; the qualitative and quantitative analysis of major sugars in honey using a novel, validated HPTLC assay.

The above assays have been used to assess more than 400 honey samples and in doing so have yielded rich data on a diverse range of Western Australian honey, providing an important first step towards the establishment of a comprehensive Australian honey library database using this technique. These analytical tests have also been used in extensive short and long-term honey stability studies, which provide important information on the impact of different handling and storage conditions on honey quality (that is, chemical composition, bioactivity).

The value of using a single analytical instrument and thus also a single lab for comprehensive honey testing has been recognised by Australia's honeybee industry, which has adopted HPTLC testing as an included methodology in its comprehensive honey quality assurance system, known as BQUAL. There is also strong international interest in these analytical capabilities, for example, from regulatory authorities such as the US Pharmacopoeia who seek to develop testing systems to authenticate honey for use in complementary medicines. The HPTLC sugar analysis developed as part of this project has since also been adopted in a comprehensive, fully automated analysis system offered by the world leader of HPTLC technology, CAMAG (based in Switzerland).

The methodologies developed in this project are also useful in the assessment of related honey bee products, such as bee pollen. They have further been used to collate data on an unusual type of 'ant honey', a culturally and nutritionally important Australian indigenous bushfood produced by so-called honeypot ants (*Camponotus inflatus*) from the secretion of sap-sucking hemipterans.

Various findings of this project have already been widely communicated in refereed academic journal articles, industry presentations and publications as well as at national and international conferences.

Academic outputs

To date (as of September 2022), ten peer-reviewed journal articles have already been published as a direct outcome of this project. At least one additional publication is already drafted, a comprehensive study on honey stability on exposure to different handling and storage conditions.

1. Peer-reviewed published papers

Islam, M.K., Lawag, I.L., Green, K.J., Sostaric, T., Hammer, K.A., Lim, L.Y. and Locher, C. 2022. An investigation of the suitability of melissopalynology to authenticate Jarrah honey, *Current Research in Food Science*, https://doi.org/10.1016/j.crfs.2022.02.014

Islam, M.K.; Vinsen, K.; Sostaric, T.; Lim, L.Y.; Locher, C. 2021. Detection of syrup adulterants in Manuka and Jarrah honey using HPTLC-multivariate data analysis. *PeerJ 9:e12186.*

Islam, M.K.; Sostaric, T., Lim, L.Y.; Hammer, K. and Locher, C. 2021. Development of an HPTLC-based dynamic reference standard for the analysis of complex natural products using Jarrah honey as a test sample. *PloS One 16:e0254857.*

Islam, M.K.; Sostaric, T.; Lim, L.Y.; Hammer, K.; Locher, C. 2021. Antioxidant HPTLC-DPPH Fingerprinting of Honeys and Tracking of Antioxidant Constituents upon Thermal Exposure. *Foods (Basel, Switzerland)* 10(2): 357.

Islam, M.K.; Sostaric, T.; Lim, L.Y.; Hammer, K.; Locher, C. 2020. Sugar Profiling of Honey for Authentication and Detection of Adulterants Using High-Performance Thin Layer Chromatography. *Molecules*, 25(22): 5289. doi: 10.3390/molecules25225289.

Islam, M.K.; Sostaric, T.; Lim, L.Y.; Hammer, K.; Locher, C. 2020. A validated method for the quantitative determination of sugars in honey using high-performance thin-layer chromatography. *JPC – Journal of Planar Chromatography – Modern TLC* 2020, 33(5): 489-499.

Islam, M.K.; Sostaric, T.; Lim, L.Y.; Hammer, K.; Locher, C. 2020. Development and validation of an HPTLC–DPPH assay and its application to the analysis of honey. *JPC – Journal of Planar Chromatography – Modern TLC* 2020, 33(3): 301-311.

Green, K. J.; Islam, M. K.; Lawag, I.; Locher, C.; Hammer, K. A., Honeys derived from plants of the coastal sandplains of Western Australia: antibacterial and antioxidant activity, and other characteristics. Journal of Apicultural Research. https://doi.org/10.1080/00218839.2022.2073953

Sindi, A.; Chawn, M.V.B.; Hernandez, M.E.; Green, K.; Islam, M.K.; Locher, C.; Hammer, K. 2019. Anti-biofilm effects and characterisation of the hydrogen peroxide activity of a range of Western Australian honeys compared to Manuka and multifloral honeys. *Scientific Reports*, 9(1): 17666.

Islam, M.K., Lawag, I.L., Sostaric, T., Ulrich, E., Ulrich, D., Dewar, T., Lim, L.Y. and Locher, C. 2022. Australian Honeypot Ant (*Camponotus inflatus*) Honey—A Comprehensive Analysis of the Physiochemical Characteristics, Bioactivity, and HPTLC Profile of a Traditional Indigenous Australian Food, *Molecules*, 27: 2154. https://doi.org/10.3390/ molecules27072154

Manuscript Under preparation

Islam, M. K.; Sostaric, T, Lim, L. Y, Hammer, K, Locher, C., A comprehensive analysis of the impacts of temperature on honey in in-process quality control and storage.

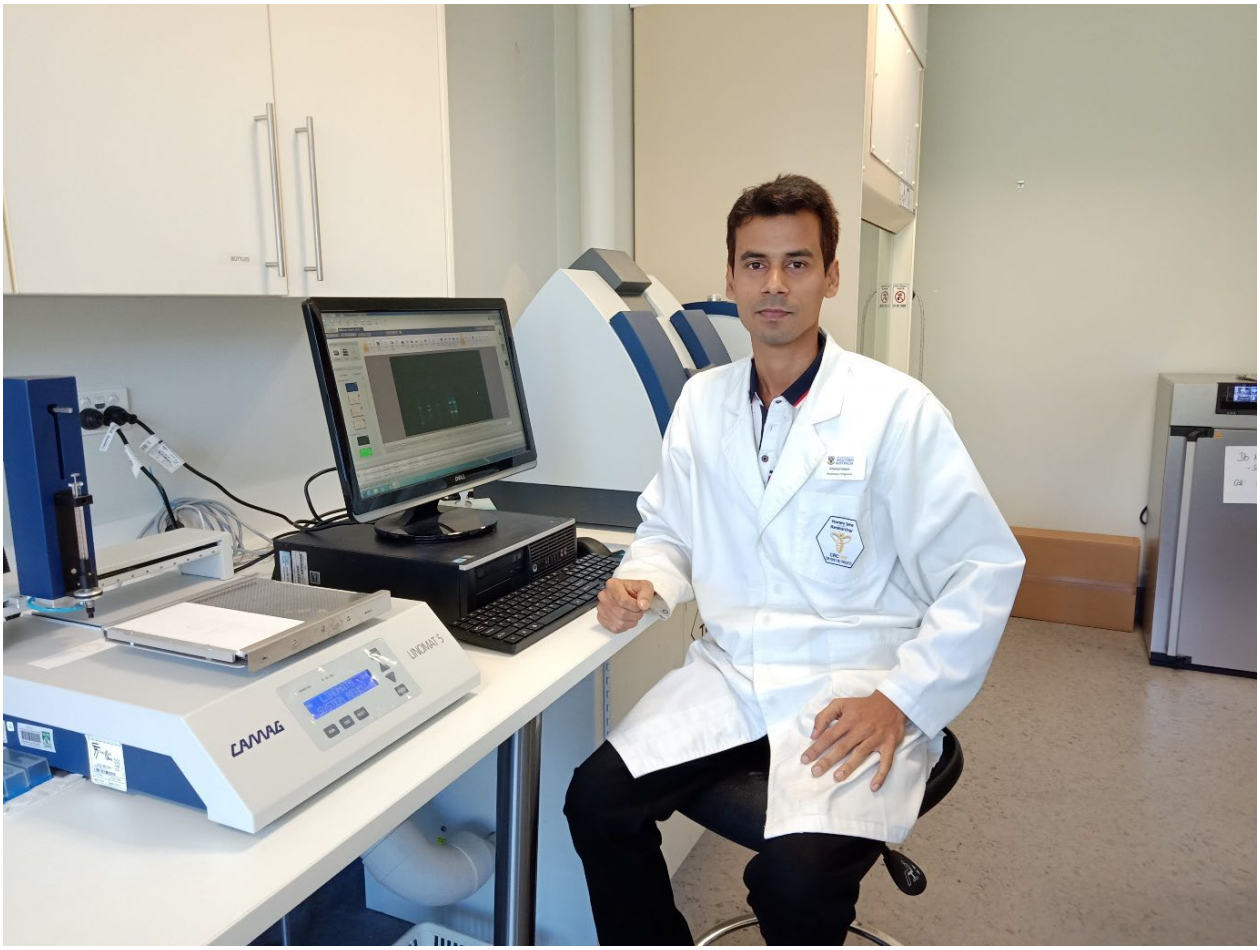

Figure 1: CRC PhD student, Md Khairul Islam, with the High-Performance Thin Layer Chromatography equipment in the University of Western Australia laboratory.

Industry outputs

For maximum impact, findings from this project have not only been presented at academic conferences but also industry workshops. A full-length article on the various HPTLC capabilities for honey analysis developed as part of this project has been published in the highly reputed industry magazine 'CAMAG Bibliography Service (CBS)' and a podcast on HPTLC fingerprinting was produced for the CRC for Honey Bee Products as part of its 'Sticky Science' Series.

Due to the industry relevance of this project, UWA Institute of Agriculture asked to present the research finding to their "2022 Postgraduate Showcase: Frontiers in Agriculture" to a large audience of academic researchers, regulatory bodies and industry stockholders.

Further evidence of industry relevance and impact is the adoption of a slightly modified (automatised) sugar HPTLC analysis method by CAMAG, the world leader in planar chromatography instrumentation, for its new HPTLC Pro System, which is based on an analysis method developed as part of this project. To promote this new methodology a webinar was organised by CAMAG catering to a global audience, which acknowledged the significant contribution to this innovation made by the research team.

Academic and industry presentations

Locher, C. 2022. HPTLC Fingerprinting for Honey Authentication. 4th Australian Bee Congress, Sydney, 8-11 June.

Islam MK, Sostaric T, Lim LY, Locher C (2022) HPTLC Analysis as a Comprehensive Authentication and Quality Control Tool for Honey – The Case Example of Western Australian Marri Honey. 25th International Symposium for High-Performance Thin-Layer Chromatography (HPTLC 2022), Ljubljana, 28 June – 1 July.

Locher, C. 2021. HPTLC for Honey Authentication. Honey Chemistry of Monoflorals Workshop, Perth, Australia, 28 October.

Islam, M. K.; Sostaric, T.; Lim, L. Y.; Hammer, K.; Locher, C. 2021. Multivariate analysis of High-Performance Thin Layer Chromatography derived data of Banksia honeys. Australasian Honey Bee Conference, Perth, Australia, 29 June – 01 July. https://www.youtube.com/watch?v=kGyHLGL4mKE

Islam, M. K.; Sostaric, T.; Lim, L. Y.; Hammer, K.; Locher, C. 2021. Tracking of thermal changes in honey using High-Performance Thin Layer Chromatography. Australasian Honey Bee Conference, Perth, Australia, 29 June – 01 July. https://www.youtube.com/watch?v=KRqJpq_YGZE

Locher, C. 2021. Exploring HPTLC as an analytical tool for BQUAL testing. BQUAL Workshop, Perth, Australia, 2 August. https://www.youtube.com/watch?v=GsZ3CGiCVrc

Islam, M.K.; Locher, C. 2019. A quick assessment of flora source. South Australian Apiarist Association Annual Meeting, Adelaide, 10-11 June.

Other outputs

Study of honeypot ants from the Goldfields reveals unique properties of honey (The West Australian, Tue 12 April 2022; https://thewest.com.au/news/kalgoorlie-miner/study-of-honeypot-ants-from-the-goldfields-reveals-unique-properties-of-honey-c-6407536?utm_source=csp&utm_medium=portal&utm_campaign=Isentia&token=2ll%2FHSepKgpYjOz2RrB5jxlofig2O7gdntk8mbVduor7HZvni8Z7eud5CUgyqGWPKVc%2BeeSF%2FXrNUSuIUCrwMA%3D%3D)

Sweet rewards from honeypot ant honey study (UWA, 5th April 2022; https://www.uwa.edu.au/news/Article/2022/April/Sweet-rewards-from-honeypot-ant-honey-study)

ABC Country Hour (5th April 2022; https://www.abc.net.au/radio/programs/wa-country-hour/wa-country-hour/13818346)

2022 Postgraduate Showcase: Frontiers in Agriculture (Institute of Agriculture, UWA; Thursday, 23 June 2022; https://www.uwa.edu.au/institutes/institute-of-agriculture/Events/2022-IOA-Postgraduate-Showcase and https://www.youtube.com/watch?v=D2dNAP54mao)

'Honey Signatures' Case Study – Translating Astronomical Research to Down-to-Earth Applications, published by the International Centre for Radio Astronomy Research, January 2022 (https://www.icrar.org/wp-content/uploads/2022/01/ICR2110-A4-Honey-Signatures_SCREEN-Spreads.pdf)

CAMAG Webcast "The Concept of Fully Automated HTPLC Analysis on the Example of Sugars in Honey" (April 2021): https://youtu.be/i265TNHIc_c

Sticky Science (Episode 4). Cooperative Research Centre for Honey Bee Products Podcast (June 2018) http://www.crchoneybeeproducts.com/sticky-science-episode-4/

Islam, M.K.; Sostaric, T.; Locher, C. 2021. Special Issue – Analysis of Honey by HPTLC. *CAMAG Bibliography Service Planar Chromatography*, 126(1): 2-7.

HPTLC Monographs for WA honeys

Monofloral Honey from *Calothamnus* spp – Red Bell Honey; Md Khairul Islam, Tomislav Sostaric and Cornelia Locher; HPTLC Atlas, HPTLC Association 2021.

Mono floral Honey from *Corymbia calophylla* – Marri Honey; Md Khairul Islam, Tomislav Sostaric and Cornelia Locher; HPTLC Atlas, HPTLC Association 2021.

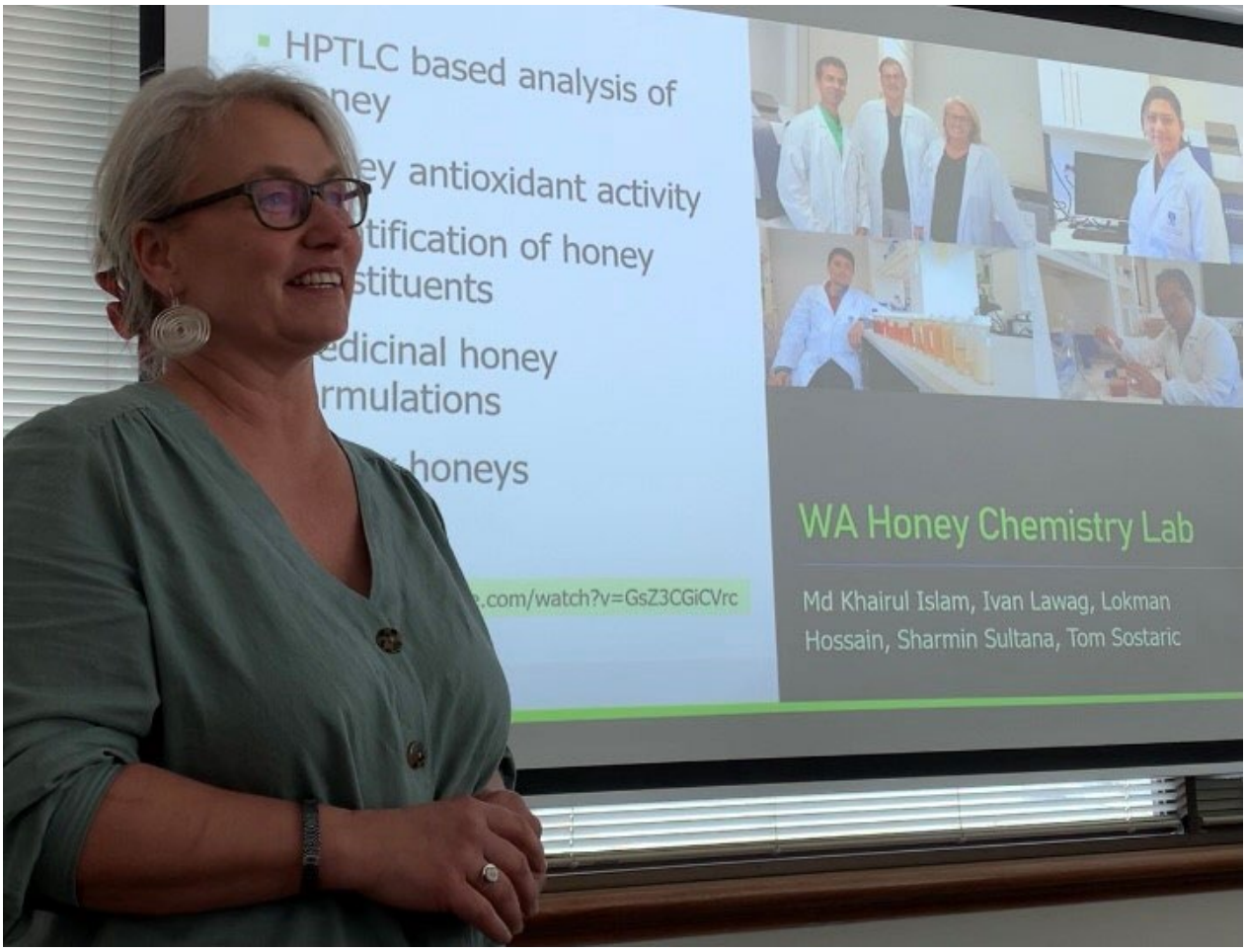

Figure 2: Project leader, A/Professor Connie Locher, presenting to the Bee Industry Council of WA.

Introduction

Operating in a biodiversity hotspot with a rich variety of often pristine endemic flora, Western Australian beekeepers produce a wide range of floral honeys. To ensure that these honeys yield market prices that reflect their uniqueness and their 'clean and green' origin, quality control becomes a crucial instrument in the industry's production processes but also as a honey marketing instrument. It is also important to collate data on the different WA honeys that are currently produced across the State to establish benchmark phytochemical and bioactivity parameters to strengthen marketing claims with scientific evidence. To assist the honey bee industry in these efforts, reliable but also convenient and cost-effective analysis techniques are required.

A wide range of methods is currently in use to monitor different aspects of honey chemistry. Physicochemical characteristics that are routinely recorded to monitor honey quality are pH and water content, with the latter being derived from the measurement of refractive index and BRIX value. The Codex Alimentarius, for instance, stipulates upper limits for moisture and sugars for a food product to be classified as 'honey' (FAOUN and WHO 2019).

The qualitative and quantitative analysis of a honey's sugar fraction is therefore an important element of analysis, which can also assist in the detection of pre- or post-harvest adulterations. The main honey sugars are fructose and glucose, alongside much smaller amounts of disaccharides (for example, sucrose, maltose, trehalose, turanose), trisaccharides (e.g., maltotriose, raffinose, erlose) and oligosaccharides (de la Fuente, 2011). The detection of sugar-based adulterations is challenging, as honey itself is a highly concentrated sugar solution, with sugars accounting for about 80–85% of the total solids in most honey. Various methods are used for honey quality control and the detection of adulterants, such as C12/C13 isotope identification, fluorescence spectroscopy, high-performance liquid chromatography (HPLC), ion exchange chromatography, gas chromatography, infrared spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and Raman spectroscopy (Rogers et al. 2014; Ventura et al. 2011; Wang et al. 2010; Schievano et al. 2013; Chen et al. 2014; Siddiqui et al. 2017). However, these methods are not without challenges (Sesta 2006; Xu et al. 2014). Sugars lack a chromophore and are poorly suited for methods that rely on detection by UV radiation. Derivatisation into more easily detectable artefacts is possible; however, these approaches might be hampered by low sensitivity and poor selectivity (Luo et al. 1993). Other methods, like IR and NMR, rely on the nonspecific detection of components and the establishment of adequate reference databases and threshold limits (Ballin and Laursen 2019; Esslinger et al. 2014).

Furthermore, the role of honey's predominant nectar source in its phytochemical and bioactivity characteristics is an important research field. This can be seen in the case of honey produced from *Leptospermum* species. *Leptospermum* nectar contains dihydroxyacetone (DHA) which is transformed into methylglyoxal (MGO) during the ripening of the honey in the hive, giving *Leptospermum* honey such as the highly prized Manuka honey, their high levels of antibacterial activity (Cokcetin et al. 2016). For many other kinds of honey, the relationship between nectar source and honey properties has not been fully explored although this might be used as an important element in the authentication of honey and the confirmation of their predominant nectar source. In recent years, High-Performance Thin Layer Chromatography (HPTLC) has been demonstrated to be a useful analytical tool for this task as an organic honey extract, when analysed with this technique, produces characteristic banding patterns reflective of the honey's nectar source. Honey of the same

floral origin produces consistent HPTLC fingerprints within natural variations, whereas those derived from different nectar sources can be discriminated against on this basis (Locher et al. 2018, 2017). It can be argued that the chemical analysis of honey, including HPTLC fingerprinting of its organic extract, which amplifies its naturally present non-sugar constituents, is superior to authentication efforts based on pollen analysis, a technique also known as melissopalynology. Pollen analysis has been used for many years to determine the botanical origin of honey, especially in Europe (Bogdanow et al. 1999; Sniderman et al. 2018; Louveaux et al. 1970). It records the type and relative quantity of different pollen grains found in honey by microscopic analysis with the dominant pollen being considered as a marker for the honey's nectar source (Lurlina et al. 2009; Jerković et al. 2009). However, while melissopalynology might be an adequate approach for many European kinds of honey a recent study by Sniderman et al. (2018) has been able to demonstrate that it is not suitable for the authentication of Australian eucalyptus honey or generally honey derived from pristine natural sources where the flowering seasons of different floral species might overlap. It also needs to be highlighted that honey is produced from flower nectar, not from flower pollen. Bees collect flower pollen to complement their nutritional needs (Zarchin et al. 2017; T'ai and Cane 2000); at times the flower pollen they collect might therefore be from a different botanical source compared to the nectar they use to produce honey. In an Australian context, melissopalynology is a controversial method of honey authentication. Furthermore, Molan (1998) pointed out that honey that has been filtered with diatomaceous earth has no pollen left that could be used for its authentication. Also, honey produced from secretions of extrafloral nectaries (a major source of honey produced from cotton plants (*Gossypium hirsutum*), castor-oil plants (*Ricinus communis*) and rubber trees (*Hevea brasiliensis*)) does not contain any pollen to identify its floral origin. Similarly, in Rewarewa (*Knightia excelsa*) and Tawari (*Lxerba brexioides*) trees endemic to New Zealand, bees don't have access to pollen during nectar collection (Li 2017), leading to an undercount of the respective pollen in the honey produced from these botanical sources. Moreover, thyme honey often has a low total pollen count as only some thyme flowers produce nectar and pollen, whereas others produce nectar only (Li 2017). Even for the well-researched Manuka honey (derived from *Leptospermum scoparium*), the limitations of melissopalynology have been demonstrated as Manuka pollen appears to be almost visually identical to Kanuka (*Kunzea ericoides*) when investigated under a light microscope (Stephens et al. 2010). Taking all of the above into account the value of melissopalynology for honey authentication might be called into question; it can be argued that the approach might be more suited to the confirmation of the honey's geographical origin and time of collection as the pollen grains present in the honey may provide evidence for many (but not necessarily all) of the floral sources the bees have visited (Robertson 2019), but it is not always a suitable tool to authenticate the nectar source of the honey.

A honey's bioactivity profile (e.g., antibacterial and/or antioxidant activity) is also an important characteristic of its quality and often a co-determinant of its market value. Maintaining the level of bioactive constituents throughout the production (collection and processing), transport and storage chain is of concern to the beekeeping industry as it will ensure the ongoing quality of their product. The antioxidant activity of honey, for example, has attracted considerable interest in recent years because of its association with anti-inflammatory, anti-cancer and anti-aging effects (e.g., Nguyen et al. 2019). It is believed to be strongly correlated to the presence of phenolic constituents (Payet et al. 2019; Singh et al. 2007; Kawabata et al. 2002), which are present in various kinds of honey in only small concentrations but contribute not only to their colour and organoleptic characteristics but also to their bioactivity profiles. The activity is commonly captured as total antioxidant activity in the so-

called FRAP (Ferric Reducing Antioxidant Power) and DPPH (2,2-Diphenyl-1-picryl-hydrazyl-hydrate) assays. A limitation of this approach is, however, that it does not allow to link the measured total antioxidant activity to the presence of compounds; honey might yield similar total antioxidant activities but differ significantly in their antioxidant constituent profiles. Identifying these key bioactive constituents should therefore become a focus of future research as it will allow their monitoring throughout the production (collection and processing), transport and storage chain to ensure a consistent quality product.

Objectives

As per the agreement, the objective of this project was to develop a real-time honey assessment tool for beekeepers and packers to be able to determine a honey's floral source alongside the collation of key phytochemical parameters for a wide range of WA honey.

In the original project agreement, the specific aims of the study were to develop:

- Potential correlations between phytochemical characteristics of WA honey and their bioactivity including the monitoring of changes over time dependent on storage and handling conditions
- The impact of DHA and MGO on hydrogen peroxide performance
- Qualitative and quantitative HPTLC fingerprinting for honey analysis

As reflected in its key activities outlined in the next section, over the lifespan of the project these objectives were refined and adjusted to better mirror current industry needs and gaps identified in the literature. In brief, the focus shifted onto the development of a range of analytical techniques, based on High-Performance Thin Layer Chromatography (HPTLC), to determine key phytochemical and bioactivity characteristics of Western Australian honey. These methods can also be used to authenticate a honey's floral origin when combined with suitable statistical analyses, and in doing so can assist quality control efforts by the Australian honeybee industry. The data generated as part of this project has since contributed to the initiation of a comprehensive Australian honey library.

Key activities

Initially, the project proposed to undertake the following activities:

1. Determine key chemical parameters for a wide range of WA honey
2. Correlate honey chemistry with bioactivity and monitor changes over time dependent on storage and handling
3. Investigate the potential impact of DHA and MGO on the presence of hydrogen peroxide and its antimicrobial performance
4. Refine qualitative and quantitative HPTLC fingerprinting as a potential new honey analysis tool

Activity #4 has been the key focus of this project and it has been possible to establish HPTLC as a novel, convenient and cost-effective honey analysis instrumentation. This is demonstrated in the development and validation of a new HPTLC-based quantification method for sugars in honey, which can be used not only to derive important honey quality parameters such as glucose, fructose, maltose and sucrose content as well as the honey's fructose to glucose ratio but also assist in the detection and quantification of post-harvest adulteration with sugar syrups, in particular when coupled with suitable statistical tools (Islam et al. 2021; Islam et al. 2020a, 2020b).

It has also been possible to develop an HPTLC-based analytical tool, which addresses a key limitation of total antioxidant activity assays so far that they are only able to capture a honey's bioactivity in its entirety without allowing insight into the individual bioactive constituents responsible for the effect. Coupling HPTLC analysis with DPPH derivatisation is an accurate and reproducible way of detecting and quantifying antioxidant honey constituents (as gallic acid equivalents), even if their chemical identity has not yet been determined (Islam et al. 2020). This novel approach provides the honeybee industry with a valuable tool to monitor honey quality throughout its production and storage (Islam et al. 2021).

This assay used only one instrumentation (i.e., HPTLC) for the comprehensive honey stability study (Activity #2). Next to potential changes to honey' antioxidant profiles, changes in sugar and HMF were also recorded on exposure to a range of temperatures (25 °C, 40 °C, 60 °C and 80 °C) over time, which reflect typical handling and storage conditions honey are exposed to (Islam et al. 2021). Data collection and analysis for this part of the project have been completed and a manuscript is ready to submit to the journal.

Activity #1 is also ongoing but has to date already been accomplished for more than 400 Western Australian honey. For all of them, (jointly with activities set out under Project 12 and Project 31/33) key Physico-chemical characteristics, such as refractive index, BRIX value, water content and pH have been determined. For all honey designated by a beekeeper to be Jarrah or Marri honey, fructose and glucose contents have been quantified and the respective fructose to glucose ratio determined. Total phenolic content was also measured for all 400+ kinds of honey. Moreover, the organic extracts of all honey were fingerprinted by HPTLC analysis to derive analytical signatures typical of the various nectar sources.

Activity #3, as highlighted in all previous quarterly reports, this activity no longer constitutes a core focus of this project as most of the Western Australian honey collected and analysed as part of this

study were not generated from the genus *Leptospermum* and therefore their dihydroxyacetone (DHA) and methyl glyoxal (MGO) content can be expected to be minimal and of no relevance for their quality.

Impacts

According to the original Project Agreement the anticipated impacts of this project were:

- Empower beekeepers and packers with knowledge of the floral source and basic phytochemical and bioactivity parameters of their honey
- Enable packers to test the impact of their handling and storage on honey quality
- Enable packers to assess the impact of blending peroxide and non-peroxide honey

In line with the key activities described above, the revised impacts of this project are:

- Empower beekeepers and packers with knowledge of the floral source and basic phytochemical and bioactivity parameters of their honey (in conjunction with Projects 12 and 31/33)
- Enable packers to authenticate honey's floral source and the detection of potential post-harvest adulteration with sugar syrups
- Enable packers to test the impact of their handling and storage on honey quality

Outputs

In the original Project Agreement, the following outputs were anticipated:

Comprehensive data on WA flora honey will be collated, including a range of phytochemical parameters and HPTLC fingerprints as a visual analysis tool, and correlated with bioactivity levels.

The impact of different storage and processing conditions on the phytochemical composition and bioactivity of these honey will be evaluated

The impact of blending peroxide and non-peroxide honey will be assessed by investigating the impact of DHA/MGO on hydrogen peroxide levels associated with antimicrobial activity

Paper 1:

Phytochemical characteristics and antimicrobial activities of a wide range of WA honey

Paper 2:

Assessment of the impact of DHA and MGO on hydrogen peroxide levels will shed more light on the benefits and obstacles associated with the blending of peroxide and non-peroxide honey

Paper 3:

HPTLC fingerprints of various WA honey as visual analysis tools

Paper 4:

Comparative analysis of HPTLC and HPLC as quantitative tools in the honey analysis

While slight adjustments had to be made to the anticipated outputs in line with shifts in key activities to date, the project has already exceeded anticipated output deliverables with the publication of ten peer-reviewed journal articles. To capture still-to-be-completed work on the honey stability study at least one additional publication is planned within the next months in addition to two more papers, one on 'WA Forest Honeys' and the other on 'WA Inland and Esperance region Honey' as joint outputs with Project 12 and Project 31/33.

Results

Determination of key chemical parameters for a wide range of WA honey

To deal with the complexities of reporting on the more than 400 honey analysed as part of this project (in conjunction with work carried out in Project 12 and Project 31/33) the various kinds of honey have been assigned to three broad groups, (1) coastal honey, (2) inland and Esperance region honey and (3) forest honey. A first manuscript, reporting findings for those honey classified as 'coastal' is currently under review. The drafting of the other two papers is currently in progress.

As an extension to this project activity, the scope of analysis has been widened and applied to a so-called 'ant honey, which is produced by Western Australian honey pot ants (*Camponotus inflatus*) from the secretion of sap-sucking hemipterans. A manuscript detailing the findings of this study is also currently under review.

- Green, K. J.; Islam, M. K.; Lawag, I.; Locher, C.; Hammer, K. A., Honeys derived from plants of the coastal sandplains of Western Australia: antibacterial and antioxidant activity, and other characteristics. *Journal of Apicultural Research*. https://doi.org/10.1080/00218839.2022.2073953

- Islam, M. K.; Lawag, I.; Sostaric, T.; Ulrich, E.; Ulrich, D.; Dewar, T.; Durman, D.; Lim, L. Y.; Locher, C., Analysis of Honey Produced by Australian Honeypot Ants (*Camponotus inflatus*). *Molecules (Basel, Switzerland)*, *27*(7), [2154]. https://doi.org/10.3390/molecules27072154

- HPTLC Fingerprints of all honey analysed to date (at 254 nm and 366 nm after development and 366 nm and white light after derivatisation with vanillin sulfuric acid reagent) (Information'Information0 honeys are available on "Chapter 2, PhD thesis _ Md Khairul Islam")

- HPTLC-based sugar analysis of selected honey blends

- HPTLC-based quantification of glucose and fructose in Jarrah and Marri honey

Correlation of honey chemistry with bioactivity and monitoring of changes over time dependent on storage and handling

The HPTLC technique was developed and applied to track the changes of physico-cheminal properties of honeys (floral fingerprints, sugar profile, DPPH band activity and formation of heat-treated artefact, 5-hydroxymethylfurfural (HMF)) over time. The results were presented as a manuscript and waiting for submission to a journal.

Islam, M. K.; Sostaric, T, Lim, L. Y, Hammer, K, Locher, C., Comprehensive HPTLC-Based Analysis of the Impacts of Temperature on the Chemical Properties and Antioxidant Activity of Honey.

Abstract: Before being sold to consumers, honeys might have already undergone a series of processing steps involving different temperatures and they might have also been stored for various lengths of time under changing ambient conditions. During post-harvest processing honey might, for example, be subjected to elevated temperatures, which could affect its physicochemical properties and bioactivity. To meet quality expectations, there is a need for robust quality control assessments during processing and during storage to ensure that exposure to higher temperatures does not compromise the honey's chemical composition and/or antioxidant activity. This paper describes a comprehensive short-term (48 h) and long-term (5 months) study of the effects of temperature (40 °C, 60 °C and 80 °C) on three commercial honeys (Manuka, Marri and Coastal Peppermint) and an artificial honey, using High Performance Thin Layer Chromatography (HPTLC) analysis. Samples were collected at baseline, 6 h, 12 h, 24 h, 48 h, and then monthly for five months and were analysed for potential changes in their organic extract HPTLC fingerprints, in their HPTLC-DPPH total band activities, in their major sugar composition and their hydroxymethylfurfural (HMF) content. It was found that while all assessed parameters changed over the monitoring period, changes were moderate at 40 °C but increased significantly with increasing temperature, especially the honeys' HPTLC-DPPH total band activity and HMF content. (Full article is available in "Chapter 5, thesis _ Md Khairul Islam")

Refinement of qualitative and quantitative HPTLC fingerprinting as a potential new honey analysis tool

The HPTLC technique was developed, and the outcomes were published in a series of papers -

Islam, M. K., Lawag, I., Green, K., Sostaric, T., Hammer, K., Lim, L. Y., & Locher, C. (2022). An investigation of the suitability of melissopalynology to authenticate Jarrah honey. Current Research in Food Science, 5, 506-514. https://doi.org/10.1016/j.crfs.2022.02.014

Abstract: This study reports on the analysis of eleven Jarrah (Eucalyptus marginata) honeys, of which nearly half (n = 5) were re-classified as Blackbutt (E. patens) honey on the grounds of the predominant flower pollen identified by melissopalynology. Based on a comprehensive analysis of the honeys' physico- and phytochemical characteristics and antioxidant activity data, taking into account pH, electrical conductivity, refractive index and Brix values as well as moisture content, individual fructose and glucose content and derived fructose to glucose ratio alongside total phenolic content and antioxidant activity determined by the DPPH assay, no statistically significant difference was found amongst the eleven honeys classified by pollen analysis into two honey groups, 'Jarrah' or 'Blackbutt'. This study therefore draws into question the value of melissopalynology as an analysis tool to authenticate Jarrah honey. (Full article is available in "Chapter 2, PhD thesis _ Md Khairul Islam" and https://doi.org/10.1016/j.crfs.2022.02.014)

Islam, M.K.; Sostaric, T., Lim, L.Y.; Hammer, K. and Locher, C. 2021. Development of an HPTLC-based dynamic reference standard for the analysis of complex natural products using Jarrah honey as a test sample. *PloS One 16:e0254857.*

Abstract: In this paper, we describe a novel approach to the development of a reference standard for the quality control of complex natural products, which will assist in the assessment of their authenticity and purity. The proposed method provides a template for the selection of samples, which can be pooled to obtain a reference standard. A shortfall of such an approach is, however, that the pooled sample is static in nature and therefore unable to capture the difference in processing conditions or natural variations triggered by geographical or climatic impacts over time. To address this, the paper also outlines the development of a dynamic reference standard, which allows for ongoing adjustments to future variations. The method employs High-Performance Thin Layer Chromatography (HPTLC) derived extract profiles processed by multivariate analysis. The development of the dynamic reference standard is illustrated using honey, a complex natural matrix, as an example. (Full article is available in "Chapter 2, PhD thesis _ Md Khairul Islam" and https://doi.org/10.1371/journal.pone.0254857)

Islam, M.K.; Sostaric, T.; Lim, L.Y.; Hammer, K.; Locher, C. 2020. A validated method for the quantitative determination of sugars in honey using high-performance thin-layer chromatography. *JPC – Journal of Planar Chromatography – Modern TLC* 2020, 33(5): 489-499.

Abstract: Sugars, in particular glucose and fructose, are the main constituents of honey, comprising up to 85% of its total weight. A high-performance thin-layer chromatography (HPTLC) method to identify and quantify common sugars (glucose, fructose and sucrose) in honey has been developed and fully validated according to the International Conference on Harmonisation guidelines. It allows for the determination of a honey's fructose-to-glucose ratio, which is not only an important authentication and quality control parameter, but also an indication of its tendency to crystallise. The HPTLC analysis is easy to perform, accurate, precise, specific and sensitive and requires only minimal sample preparation. With a limit of detection/limit of quantification of 21.98 ng/66.62 ng for fructose, 33.00 ng/100.00 ng for glucose and 21.17 ng/64.15 ng for sucrose, the sensitivity of the method has been greatly improved compared to other HPTLC-based approaches. An additional advantage is the method's simplicity and fast processing time as it only requires a single development step without a plate or sample pre-treatment. (Full article is available in "Chapter 3, PhD thesis _ Md Khairul Islam" and https://doi.org/10.1007/s00764-020-00054-9)

Islam, M.K.; Sostaric, T.; Lim, L.Y.; Hammer, K.; Locher, C. 2020. Sugar Profiling of Honeys for Authentication and Detection of Adulterants Using High-Performance Thin Layer Chromatography. *Molecules*, 25(22): 5289. doi: 10.3390/molecules25225289.

Abstract: Honey adulteration, where a range of sugar syrups is used to increase bulk volume, is a common problem that has significant negative impacts on the honey industry, both economically and from a consumer confidence perspective. This paper investigates High-Performance Thin Layer Chromatography (HPTLC) for the authentication and detection of sugar adulterants in honey. The sugar composition of various Australian honeys (Manuka, Jarrah, Marri, Karri, Peppermint and White Gum) was first determined to illustrate the variance depending on the floral origin. Two of the honeys (Manuka and Jarrah) were then artificially adulterated with six different sugar syrups (rice, corn, golden, treacle, glucose and maple syrup). The findings demonstrate that HPTLC sugar profiles, in combination with organic extract profiles, can easily detect sugar adulterants. As major sugars are found in honey, the quantification of fructose and glucose, and their concentration ratio can be used to authenticate the honeys. Quantifications of sucrose and maltose can be used to identify the type of syrup adulterant, when used in combination with HPTLC fingerprinting of the organic honey extracts. (Full article is available in "Chapter 3, PhD thesis _ Md Khairul Islam" and doi:10.3390/molecules25225289)

Islam, M.K.; Vinsen, K.; Sostaric, T.; Lim, L.Y.; Locher, C. 2021. Detection of syrup adulterants in Manuka and Jarrah honey using HPTLC-multivariate data analysis. *PeerJ 9:e12186.*

Abstract: High-Performance Thin-Layer Chromatography (HPTLC) was used in a chemometric investigation of the derived sugar and organic extract profiles of two different honeys (Manuka and Jarrah) with adulterants. Each honey was adulterated with one of six different sugar syrups (rice,

corn, golden, treacle, glucose and maple syrups) in five different concentrations (10%, 20%, 30%, 40%, and 50% w/w). The chemometric analysis was based on the combined sugar and organic extract profiles' datasets. To obtain the respective sugar profiles, the amount of fructose, glucose, maltose, and sucrose present in the honey was quantified and for the organic extract profile, the honey's dichloromethane extract was investigated at 254 and 366 nm, as well as at T (Transmittance) white light and 366 nm after derivatisation. The presence of sugar syrups, even at a concentration of only 10%, significantly influenced the honeys' sugar and organic extract profiles and multivariate data analysis of these profiles, in particular cluster analysis (CA), principal component analysis (PCA), principal component regression (PCR), partial least-squares regression (PLSR) and Machine Learning using an artificial neural network (ANN), were able to detect post-harvest syrup adulterations and to discriminate between neat and adulterated honey samples. Cluster analysis and principal component analysis, for instance, could easily differentiate between neat and adulterated honeys using CA or PCA plots. In particular, the presence of excess amounts of maltose and sucrose allowed for the detection of sugar adulterants and adulterated honeys by HPTLC-multivariate data analysis. Partial least-squares regression and artificial neural networking were employed, with augmented datasets, to develop optimal calibration for the adulterated honeys and to predict those accurately, which suggests a good predictive capacity of the developed model. (Full article is available in "Chapter 3, PhD thesis _ Md Khairul Islam" and https://peerj.com/articles/12186/)

Islam, M.K.; Sostaric, T.; Lim, L.Y.; Hammer, K.; Locher, C. 2020. Development and validation of an HPTLC–DPPH assay and its application to the analysis of honey. *JPC – Journal of Planar Chromatography – Modern TLC* 2020, 33(3): 301-311.

Abstract: Using honey as a model sample, this study aims to develop and validate a simple, rapid screening tool that allows for the visualization of constituents that contribute to the antioxidant activity of a complex natural product and to quantify their individual antioxidant effects even if their chemical identity is unknown. The method employs a validated analysis, which is based on the separation of constituents using high-performance thin-layer chromatography (HPTLC) followed by their visualization using DPPH* (2,2-diphenyl-1-picrylhydrazyl) as derivatizing reagent and the quantification of the antioxidant activity of individual bands expressed as gallic acid equivalents. (Full article is available in "Chapter 4, PhD thesis _ Md Khairul Islam" and https://link.springer.com/article/10.1007/s00764-020-00033-0)

Islam, M.K.; Sostaric, T.; Lim, L.Y.; Hammer, K.; Locher, C. 2021. Antioxidant HPTLC-DPPH Fingerprinting of Honeys and Tracking of Antioxidant Constituents upon Thermal Exposure. *Foods (Basel, Switzerland)* 10(2): 357.

Abstract: The use of High-Performance Thin-Layer Chromatography (HPTLC) coupled with the use of DPPH* (2,2-diphenyl-1-picrylhydrazyl) as a derivatisation reagent is a novel approach to the analysis of the antioxidant activity of honeys. The method facilitates the visualisation of individual constituents that contribute to the overall antioxidant activity of the honey, even if they are not yet chemically identified, and allows for the quantification of their antioxidant activity as gallic acid equivalents. The method supports a more in-depth study of the antioxidant activity of honey as it

allows for a comparative analysis of the antioxidant fingerprints of honeys of different floral origins and can capture differences in their bioactive constituents. Further, it supports the tracking of changes in antioxidant activity of individual honey constituents over time upon exposure to different temperature conditions, which demonstrates the potential value of the method for in-process quality control. (Full article is available in "Chapter 4, PhD thesis _ Md Khairul Islam" and https://doi.org/10.3390/foods10020357)

Recommendations

This project has successfully completed its key objectives. There are some further recommendations for the honeybee industry.

As a part of this research project, several HPTLC-based analytical tools were developed. But those tools needed to be commercially available for the beekeepers to use cost-effectively. We believe those analytical tools will help the honeybee industry to value-add their products through proper identification of the floral source, detecting the adulteration and finally determining the bioactivity of their products.

The analyse of the nectar of flowers by the HPTLC technique and to evaluate potential correlations between the flower nectar fingerprints and the corresponding honey fingerprints as a further confirmation of the suitability of HPTLC analysis for the authentication of a honey's nectar origin. Future research can focus on these aspects.

To authenticate the floral source of a particular honey, a comparator or standard is needed. The data generated from this project could form the basis for the development of HPTLC Monographs for the WA honeys. Already two HPTLC monographs (e.g., Marri and Red bell) have developed and published. It is recommended that future study can focus on the other iconic WA honeys.

References

Ballin, N.Z.; Laursen, K.H. 2019. To target or not to target? Definitions and nomenclature for targeted versus non-targeted analytical food authentication. Trends Food Sci. Technol., 86, 537–543.

Bogdanov, S., et al. 1999. Honey quality and international regulatory standards: review by the International Honey Commission. Bee World, 80(2): 61-69.

Chen, Q.; Qi, S.; Li, H.; Han, X.; Ouyang, Q.; Zhao, J. 2014. Determination of rice syrup adulterant concentration in honey using three-dimensional fluorescence spectra and multivariate calibrations. Spectrochim. Acta A Mol. Biomol. Spectrosc., 131, 177–182.

Cokcetin, N.N.; Pappalardo, M.; Campbell, L.T.; Brooks, P.; Carter, D.A.; Blair, S.E.; Harry, E.J. 2016. The antibacterial activity of Australian *Leptospermum* honey correlates with methylglyoxal levels. PLoS One, 11(12): e0167780.

de la Fuente, E.; Ruiz-Matute, A.I.; Valencia-Barrera, R.M.; Sanz, J.; Castro, I.M. 2011. Carbohydrate composition of Spanish unifloral honeys. Food Chem., 129, 1483–1489.

Esslinger, S.; Riedl, J.; Fauhl-Hassek, C. 2014. Potential and limitations of non-targeted fingerprinting for authentication of food in official control. Food Res. Int., 60, 189–204.

Food and Agricultural Organization of the United Nations (FAOUN) and World Health Organisation (WHO) 2019. *Codex Alimentarius. The standard for Honey.* Available at http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B12-1981%252FCXS_012e.pdf.

Islam, M. K.; Sostaric, T.; Lim, L. Y.; Hammer, K.; Locher, C. 2020a. Sugar Profiling of Honeys for Authentication and Detection of Adulterants Using High-Performance Thin Layer Chromatography. *Molecules* 25(22): 5289. doi: 10.3390/molecules25225289.

Islam, M. K.; Sostaric, T.; Lim, L. Y.; Hammer, K.; Locher, C. 2020b. A validated method for the quantitative determination of sugars in honey using high-performance thin-layer chromatography. JPC – Journal of Planar Chromatography – Modern TLC, 33(5), 489-499.

Islam, M. K.; Vinsen K.; Sostaric T.; Lim L.Y.; Locher C. 2021. Detection of syrup adulterants in Manuka and Jarrah honey using HPTLC-multivariate data analysis. PeerJ 9:*e12186.*

Islam, M.K.; Sostaric, T.; Lim, L.Y.; Hammer, K.; Locher, C. 2020. Development and validation of an HPTLC–DPPH assay and its application to the analysis of honey. JPC – Journal of Planar Chromatography – Modern TLC, 33(3): 301-311.

Islam, M.K.; Sostaric, T.; Lim, L.Y.; Hammer, K.; Locher, C. 2021. Antioxidant HPTLC-DPPH Fingerprinting of Honeys and Tracking of Antioxidant Constituents upon Thermal Exposure. Foods, 10(2): 357.

Iurlina, M.O.; Saiz, A.I.; Fritz, R.; Manrique, G.D. 2009. Major flavonoids of Argentinean honeys. Optimisation of the extraction method and analysis of their content in relationship to the geographical source of honeys. Food Chemistry, 115(3): 1141-1149.

Jerković, I.; Tuberoso, C.I.G.; Marijanovic, Z.; Jelic, M.; Kasum, A. 2009. Headspace, volatile and semi-volatile patterns of *Paliurus spina-christi* unifloral honey as markers of botanical origin. Food Chemistry, 112(1): 239-245.

Kawabata, J.; Okamoto, Y.; Kodama, A.; Makimoto, T.; Kasai, T. 2002. Oxidative dimers produced from protocatechuic and gallic esters in the DPPH radical scavenging reaction. J Agric Food Chem, 50(19): 5468-5471. doi:10.1021/jf020347g.

Newstrom-Lloyd, L.; Raine, I.; Li, X. 2017. Pondering over and under pollen representation in nectar. New Zealand Beekeeper, 25(10): 25-27.

Locher, C.; Neumann, J.; Sostaric, T. 2017. Authentication of honeys of different floral origins via HPTLC fingerprinting. Journal of Planar Chromatography – Modern TLC, 30(1): 57-62.

Locher, C.; Tang, E.; Neumann, J.; Sostaric, T. 2018. High-Performance Thin-Layer Chromatography Profiling of Jarrah and Manuka Honeys. Journal of Planar Chromatography – Modern TLC, 31(3): 181-189.

Louveaux, J.; Maurizio, A.; Vorwohl, G. 1970. Methods of Melissopalynology. Bee World, 51(3): 125-138.

Luo, P.; Luo, M.Z.; Baldwin, R.P. Determination of sugars in food products: Using HPLC and electrochemical detection at a Cu electrode. J. Chem. Educ. 1993, 70, 679.

Molan, P.C. 1998. The limitations of the methods of identifying the floral source of honeys. Bee World, 79(2): 59-68.

Nguyen, H.T.L.; Panyoyai, N.; Kasapis. S.; Pang, E.; Mantri, N. 2019. Honey and its role in relieving multiple facets of atherosclerosis. Nutrients, 11(1): 167; https://doi.org/10.3390/nu11010167.

Payet, B.; Shum Cheong Sing, A.; Smadja, J. 2005. Assessment of antioxidant activity of cane brown sugars by ABTS and DPPH radical scavenging assays: determination of their polyphenolic and volatile constituents. J Agric Food Chem, 53(26): 10074-10079. doi:10.1021/jf0517703.

Robertson, K. 2019. Understanding the value of pollen counting. New Zealand Beekeeper, 27(3): 47-51.

Rogers, K.M.; Sim, M.; Stewart, S.; Phillips, A.; Cooper, J.; Douance, C.; Pyne, R.; Rogers, P. 2014. Investigating C-4 Sugar Contamination of Manuka Honey and Other New Zealand Honey Varieties Using Carbon Isotopes. J. Agric. Food Chem., 62, 2605–2614.

Schievano, E.; Morelato, E.; Facchin, C.; Mammi, S. 2013. Characterization of markers of botanical origin and other compounds extracted from unifloral honeys. J. Agric. Food Chem., 61, 1747–1755.

Sesta, G. 2006. Determination of sugars in royal jelly by HPLC. Apidologie, 37, 84–90.

Siddiqui, A.J.; Musharraf, S.G.; Choudhary, M.I.; Rahman, A.-U. 2017. Application of analytical methods in authentication and adulteration of honey. Food Chem., 217, 687–698.

Singh, N.; Sharma, R.; Balapure, A.K. 2007. pH-regulated scavenging activity of beer antioxidants through modified DPPH assay. Toxicol Ind Health, 23(2): 75-81. doi:10.1177/0748233707077429.

Sniderman, J.M.K.; Matley, K.A.; Haberle, S.G.; Cantrill, D.J. 2018. Pollen analysis of Australian honey. PLOS ONE, 13(5): e0197545.

Stephens, J.M.; Schlothauer, R.C.; Morris, B.D.; Yang, D.; Fearnley, L.; Greenwood, D.R.; Loomes, K.M. 2010. Phenolic compounds and methylglyoxal in some New Zealand manuka and kanuka honeys. Food Chemistry, 120(1): 78-86.

T'ai, H.R.; Cane, J.H. 2000. Pollen nutritional content and digestibility for animals. Pollen and Pollination, 187-209.

Ventura, E.E.; Davis, J.N.; Goran, M.I. 2011. The sugar content of popular sweetened beverages based on objective laboratory analysis: Focus on fructose content. Obesity, 19, 868–874.

Wang, J.; Kliks, M.M.; Jun, S.; Jackson, M.; Li, Q.X. 2010. Rapid analysis of glucose, fructose, sucrose, and maltose in honeys from different geographic regions using Fourier transform infrared spectroscopy and multivariate analysis. J. Food Sci., 75, C208–C214.

Xu, W.; Liang, L.; Zhu, M. 2014. Determination of Sugars in Molasses by HPLC Following Solid-Phase Extraction. Int. J. Food Prop., 18, 547–557.

Zarchin, S.; Dag, A.; Salomon, M.; Hendriksma, H.P.; Shafir, S. 2017. Honey bees dance faster for pollen that complements colony essential fatty acid deficiency. Behavioural Ecology and Sociobiology, 71(12): 172.

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