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# Determination of authenticity of Manuka honey by MALDI-TOF mass spectrometry

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## 1. Introduction

Manuka honey is produced by bees using nectar from the Manuka tree *Leptospermum scoparium* (Figure 1). There are reported health benefits and qualities associated with Manuka honey including antimicrobial activity, antioxidant and anti-inflammatory properties. Given the criteria that must be met in order for a honey product to qualify as 'Manuka honey', the number of producers and yield of Manuka honey is lower than that of regular non-Manuka honey. As a result, Manuka-certified honey products attract a price-premium making them obvious targets for adulteration and misrepresentation. MALDI-TOF mass spectrometry has several advantages for high-throughput screening applications. Here, we evaluate for the first time the use of MALDI-TOF MS to detect and characterise key Manuka honey markers allowing the differentiation of Manuka/non-Manuka products.

Figure 1. *Leptospermum scoparium* flower [1].

## 2. Methods and Materials

Honey products labelled as 'Manuka' were purchased from local supermarkets. In order to detect the polyphenols, including the unique Manuka honey markers, the samples were subjected to SPE extraction to remove the abundant sugar component. Using Strata-X polymeric reversed phase (RP) SPE cartridges (Phenomenex), the extraction procedure was optimised allowing efficient enrichment of the non-polar components which were subsequently analysed using 2,4,6-trihydroxyacetophenone (THAP) MALDI matrix containing 10 mM Sodium Trifluoroacetate (NaTFA) in positive ion mode on a benchtop linear MALDI-TOF mass spectrometer (MALDI-8030, Shimadzu; Figure 2). The tentative MS-based assignments from the linear mode analysis were confirmed by high-energy CID MALDI-MS/MS analysis acquired on a MALDI-7090™ MALDI-TOF/TOF mass spectrometer (Shimadzu; Figure 2). The MS/MS spectra for the detected components were compared with MS/MS spectra obtained for the corresponding standards.

## 3. Result

### 3-1. MALDI-MS

Preliminary tests on direct analysis of honey solutions without any pre-treatment resulted in the detection of intense signals corresponding to the abundant sugar components present in the honey which mask/suppress the lower abundance target peaks of interest. Using the polymeric RP Strata-X SPE cartridges, the washing and elution steps were optimised, resulting in significantly cleaner MALDI spectra allowing the detection of several key polyphenols. Figure 3 shows the MALDI-MS profiles of the polyphenols after SPE enrichment of a blossom (non-Manuka) and Manuka 100+ MGO honey (Figure 3; blue and red traces, respectively).

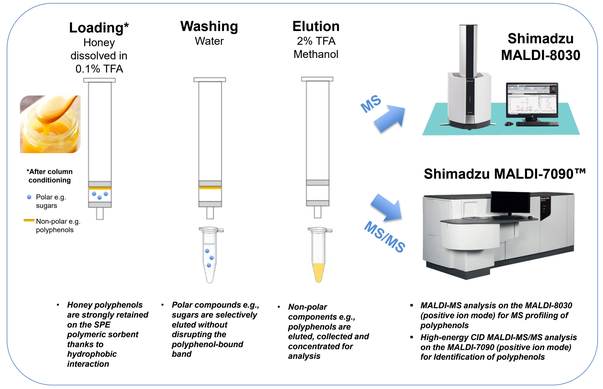


Figure 2. Sample preparation and analysis workflow for the profiling and identification of honey polyphenols.

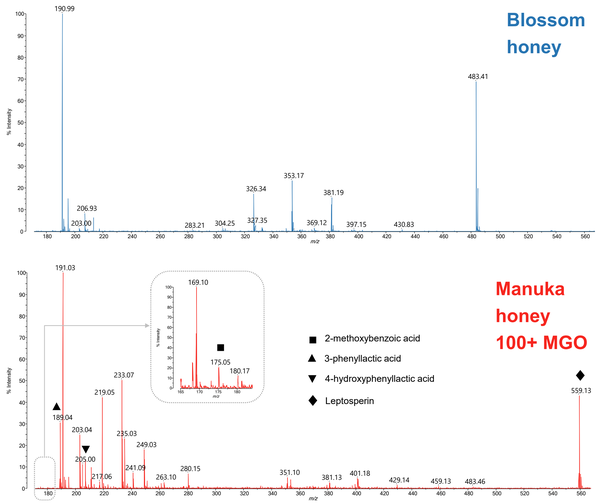


Figure 3. MALDI-MS profiles of the polyphenols after SPE enrichment of a blossom (non-Manuka) honey (blue trace) and a Manuka 100+ MGO honey (red trace). Key markers of Manuka honey, including the leptosperin (a marker for authenticity [2]) are detected in the Manuka honey sample.

As it can be seen from the Manuka honey profile, the leptosperin (a known Manuka honey marker for authenticity [2]) was clearly detected at *m/z* 559 ([M + Na]$^+$), along with other known markers such as 4-hydroxyphenyllactic acid (*m/z* 205; [M + Na]$^+$), 3-phenyllactic acid (*m/z* 189; [M + Na]$^+$), and 2-methoxybenzoic acid (*m/z* 175; [M + Na]$^+$), providing a means for the classification of Manuka/non-Manuka honey products. In the blossom honey profile, none of Manuka honey markers are detected, as expected. Other minor flavonoid components were also detected among which were chrysin (*m/z* 277; 1), pinocembrin (*m/z* 279; 2), apigenin (*m/z* 293; 3), pinobanksin (*m/z* 295; 4), luteolin (*m/z* 309; 5) and 8-methoxykaempferol (*m/z* 339; 6) (Figure 4).

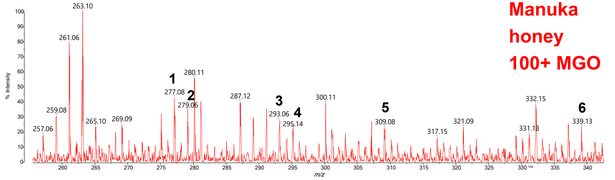


Figure 4. Expansion of the MALDI-MS spectrum of the Manuka 100+ MGO honey in the flavonoid region. 1: chrysin. 2: pinocembrin. 3: apigenin. 4: pinobanksin. 5: luteolin. 6: 8-methoxykaempferol.

### 3-2. High-energy CID MALDI-MS/MS

The unique Manuka honey markers were identified based on comparison of their MS/MS spectra with those of commercial standards. Figure 5 shows the identification of the 3-phenyllactic acid marker (*m/z* 189). The similarity between the spectra of the Manuka honey (red trace) and standard (blue trace in insert) is significant. In the absence of a commercially available leptosperin standard, the identity was confirmed by identification of peaks in the MS/MS spectrum that were consistent with the known structure of leptosperin (Figure 6). This, along with the fact that it is an expected component of the Manuka honey, allowed us to identify this component with a good degree of confidence.

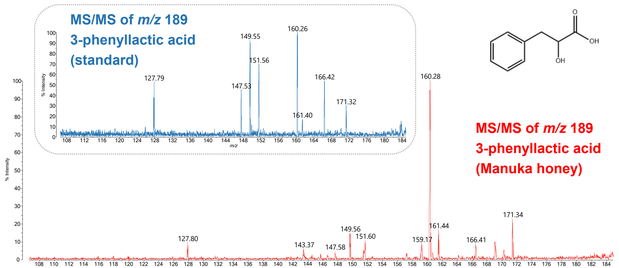


Figure 5. High-energy CID MALDI-MS/MS spectrum of *m/z* 189 (3-phenyllactic acid). Red trace: 3-phenyllactic acid in Manuka honey sample. Blue trace in insert: 3-phenyllacid acid commercial standard.

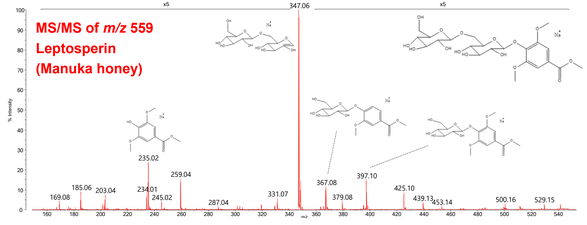


Figure 6. High-energy CID MALDI-MS/MS spectrum of *m/z* 559 (leptosperin). In the absence of a commercial standard, the identity was confirmed by identification of key fragments based on the known structure of leptosperin.

The minor flavonoid components were identified based on comparison to the MS/MS spectrum of chrysin standard (pinocembrin, pinobanksin and the other flavonoids are structurally-related analogues of chrysin with different levels of saturation/unsaturation and presence and number of hydroxyl groups). Figure 7 shows an example of identification of pinocembrin (*m/z* 279) in the Manuka honey sample (red trace) based on the MS/MS spectrum of chrysin standard (*m/z* 277; blue trace in insert): common fragment ions were detected with a +2 Da difference in the pinocembrin spectrum (the B ring in pinocembrin is saturated).

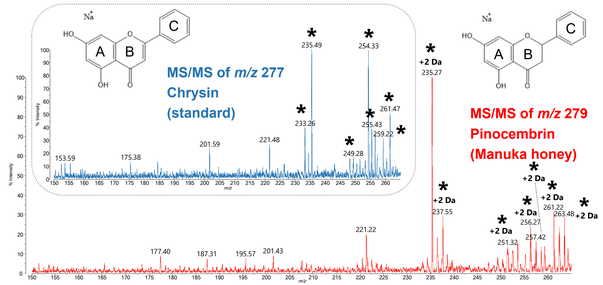


Figure 7. Identification of pinocembrin in Manuka honey (*m/z* 279; red trace) based on chrysin standard (*m/z* 277; blue trace) by high-energy CID MALDI-MS/MS. Common fragment ions were detected with a +2 Da difference in the pinocembrin spectrum (the B ring in pinocembrin is saturated).

## 4. Conclusions

This work demonstrates the usefulness of MALDI-TOF mass spectrometry to determine the authenticity of Manuka honey through detection of key markers. We hope the proposed new workflow can be used to rapidly screen the authenticity of Manuka honey products.

## References

[1] https://en.wikipedia.org/wiki/Leptospermum_scoparium
[2] https://www.umf.org.nz/unique-manuka-factor/