Ministry for Primary Industries
Manatū Ahu Matua

Science and characterising mānuka honey

Current and future science to support a definition

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Contents

1 Summary

Developing a robust definition for mānuka honey requires the identification of characteristics unique to this type of honey. These characteristics must be consistently identifiable, relatively easy and cost-effective to measure and ideally stable for the shelf life of the product. While a large amount of data was available and has been analysed, this has been diverse and mostly not robust enough for unique, stable mānuka identifiers to be established.

MPI has therefore identified the need for further science to establish appropriate characteristics and associated testing for mānuka honey. In the interim, MPI has developed a set of high level characteristics for mānuka-type honey using information currently available.

MPI has initiated several projects that will scope further science required. MPI is managing this science programme, in consultation with industry and other experts. This programme will deliver robust, usable and independently validated outcomes. These outcomes will form the basis for characterising monofloral and multifloral mānuka honey.

2 Context

MPI has been working closely with representatives and scientists associated with the New Zealand mānuka honey industry to develop an interim labelling guide. The labelling guide follows a two staged process. The first stage focuses primarily on addressing labelling issues, including health and therapeutic claims made in association with mānuka honey. The first stage also introduces some high level characteristics that help to characterise mānuka-type honey. The second stage will be to characterise and distinguish mānuka honey with an increased level of confidence. To achieve this, a robust and independent research programme is required.

This document describes:

- the analysis conducted by MPI on various honey datasets to establish the high level characteristics for mānuka-type honey;
- the MPI-funded pilot research projects that help scope suitable characteristics for additional science projects; and
- the future science required to characterise both monofloral and multifloral mānuka honey.

3 Analysis of honey testing data

3.1 BACKGROUND

During the development of the interim guide, MPI had the opportunity to analyse data supplied by the New Zealand mānuka honey industry and associated researchers. The intention was to see if it was possible to define monofloral and multifloral characteristics using existing information.

Despite the vast amount of information provided, it became evident that there are very different approaches to honey testing within industry. This meant that there was very little overlap between similar types of information from different companies. In addition, missing information within the datasets meant they lacked the robustness necessary for scientific validation. This presented significant challenges when trying to determine what characteristics and associated ranges or levels were appropriate for defining mānuka-type honey, particularly monofloral honey.

MPI explored a variety of approaches to try and resolve these variations, including a statistical model. During the assessment of the suitability of the statistical approach, a total of 18 datasets were used by MPI (sources include industry, research providers and data generated by MPI). Information on various parameters were evaluated to identify which characteristics were most useful for defining mānuka-type honey (monofloral and multifloral).

Limitations associated with the data were apparent including variations in parameters measured, incomplete data and missing information on provenance, honey type, collection year and geographic origin. These limitations presented too much uncertainty to provide confidence for a monofloral mānuka definition so it was deemed appropriate to identify more general characteristics of a mānuka-type honey.

3.2 ESTABLISHING CHARACTERISTICS FOR MANUKA TYPE HONEY

Initial data analysis considered over 11,000 honey samples and represented honey labelled as mānuka and non-mānuka from both the North and South Islands of New Zealand. The model was used to determine whether a honey sample could be classified as mānuka-type or not, using a set of measured variables. Colour, conductivity, percentage of mānuka type pollen and total mānuka-type pollen were identified from the model as useful parameters for characterising mānuka-type honey. These parameters were then tested against other datasets for which similar information was available. This was done to determine how appropriate they were for classifying mānuka and non-mānuka honey types. Methylglyoxal (MG) and dihydroxyacetone (DHA) parameters for the 11, 000 honey samples were not available.

From the analysis conducted, colour and conductivity ranges were identified that could be applied to mānuka-type honey, whilst noting that some other honey types would also meet this criteria.

In order to further distinguish mānuka-type honey from other honey types, levels of mānuka-type pollen, MG and DHA were investigated. However, in addition to the data challenges, these parameters raised questions about their suitability for definitively characterising mānuka honey, particularly monofloral honey.

Historically, pollen counts performed on mānuka-type honey recognise that more than one species of plant is represented. These species are *Leptospermum scoparium* and *Kunzea ericoides*. Given that these two plants have different characteristics, their influence on the various characteristics of mānuka-type honey may be important. Therefore, specifying a level

of pollen in the interim guide that encompasses two species of plant poses a number of challenges. MPI is assessing the feasibility and importance of being able to separate the two species from both a scientific and regulatory perspective.

DHA, from nectar, is chemically converted to MG during the production of honey in the hive. The levels of these two naturally occurring chemicals will change over time. Varying ratios between DHA and MG during the life of the honey are recognised, but are insufficiently defined to cover honey from all regions and ages. In general, a young honey will have a higher level of DHA compared to MG, and as time progresses the level of MG increases and DHA decreases. Therefore, specifying a required level for chemicals in the labelling guide that change over time needs further investigation. Also, the reported uniqueness of these chemicals to *L. scoparium* needs consideration. MPI is assessing the suitability of these chemicals from both a scientific and regulatory perspective.

Recognising the limitations of the data and information available, MPI has developed a set of high level characteristics for mānuka-type honey (refer Interim Labelling Guide for Mānuka Honey). These characteristics are recognised as interim and it is acknowledged that the outcomes of current and future MPI funded science will be used for further refinement.

4 Preliminary science projects

MPI has initiated several scoping research projects. These projects will define areas of science suitable for further development and validation. The outcomes of the validated science will help refine characteristics for both monofloral and multifloral mānuka honey and support product certification.

MPI has engaged several service providers to provide preliminary science on:

- pollen characteristics and methodology;
- genetic markers; and
- chemical markers.

Science projects completed at the end of June, 2014 are outlined below.

4.1 POLLEN ANALYSIS

4.1.1 Microscopy

- Preliminary assessment of standard microscopy to differentiate *L. scoparium* and *K. ericoides* pollen using pollen reference material.
- Investigate differences between male and hermaphrodite *L. scoparium* and *K. ericoides* pollen.
- Pollen enumeration.
- Standardisation of methodology for the extraction and enumeration of pollen.
- Classifynder – use of an automated pollen analysis instrument to differentiate *L. scoparium* and *K. ericoides* pollen.

4.2 GENETIC (DNA) ANALYSIS

4.2.1 Quantitative real time PCR

- Identification of DNA markers specific to *L. scoparium* and *K. ericoides*.
- Development and optimisation of honey DNA extraction technique suitable for high throughput.
- Development and initial validation of quantitative real-time PCR to determine the amount of *L. scoparium* and *K. ericoides* DNA in a honey sample.

4.3 CHEMICAL ANALYSIS

- Colour;
- Conductivity;
- C_3:C_4 Sugars;
- Moisture;
- HMF;
- DHA;
- MGO; and
- Chemical fingerprinting.

The over-arching objective of these projects is to conduct as many different tests as possible on the same panel of honey samples to enable comparisons to be made.

Honey samples (approximately 50) include honey believed to be:

- mainly *L. scoparium*;
- mainly *K. ericoides*; and,
- other floral type honey (including honey from Australia).

4.4 PRELIMINARY RESULTS

4.4.1 Pollen analysis

Initial results of the pollen projects are promising with clear, though subtle morphological differences between *L. scoparium* and *K. ericoides* being determined through microscopic measurement. Preliminary results suggest that these differences can be identified both by using direct light microscopy and Classifynder. Male and hermaphrodite aspects do not appear to confound pollen measurements that are used to separate the two species. However, further specificity assessment, influence of geographic variations, robust validation and suitability assessment will be required before a morphological technique could be used to characterise mānuka honey.

4.4.2 Genetic (DNA) analysis

The ability to differentiate *L. scoparium* and *K. ericoides* is also possible using a DNA approach. The preliminary results of this work show differentiation is possible. Initial test validation suggests a high level of specificity as the test does not detect other related species of concern (e.g. Jellybush). The test has also been developed to consider automated, high-throughput sample processing and testing. This will increase the number of samples that can be processed and reduce testing costs for industry. However, further work is required to refine and validate this approach so that it can be used as a robust and reliable quantitative test.

4.4.3 Chemical analysis

Chemical analysis conducted on the honey samples has identified a variety of parameters and quantifiable chemicals that may be further explored. There has been extensive research on this within the industry, and the MPI project built on this. Potential characterising compounds have been identified and others will continue to be investigated. Like the pollen and DNA analysis, further validation is required. This is needed to determine the specificity of the chemicals identified across a wide range of plant and honey types, regions and years. This type of testing also lends itself to automation and high throughput capacity.

5 Future Science

The science programme to further characterise mānuka honey will focus on both monofloral and multifloral characteristics. The programme will be heavily influenced by the appropriateness of the target characteristic(s) for test accreditation and product assurance.

Various aspects will be considered during the science programme.

These include:

- the suitability of the science for product assurance;
- the suitability of the science for validation (e.g. specificity, sensitivity, repeatability, and reproducibility);
- the long term stability of science, that is, will the science remain robust?
- the ability to provide confidence to consumers;
- the ease of technology transfer and ease of uptake by others (New Zealand and International);
- the associated testing costs and required time efficiencies; and
- the acceptability by overseas regulators and authorities.

Preliminary results from the pilot projects support further assessment and validation of one or more of the initial approaches (e.g. pollen, DNA, chemical). However, in addition to developing and validating the laboratory test for a specific characteristic(s), other aspects will also need to be considered.

These include:

- provenance of honey samples for the assessment of the selected laboratory tests;
- sampling protocols to ensure test results are representative of the batch of honey;
- the influence of honey extraction procedures on the target characteristic;
- geographic and seasonal differences;
- stability of the characteristic over the life of the product; and,
- the influence of other similar honey types e.g. other *Leptospermum* honeys.

A substantial independent science programme is required to generate outcomes that will meet the needs for all stakeholders and provide confidence to consumers and overseas regulators. It is expected that this programme would span 1-2 years and be jointly funded by MPI and industry. The science generated by this programme will undergo independent peer-review and be published.

MPI will work and consult with industry to scope, develop and deliver the required science programme.