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The rapid evaporative ionisation mass spectrometry metabolite fingerprint of *Leptospermum* honey is strongly associated with geographic origin

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ABSTRACT

There is debate about whether the honey from *Leptospermum scoparium* nectar in New Zealand is substantially different to honey from *Leptospermum* spp nectar from Australia; many *Leptospermum* honeys have high amounts of the antibacterial compound methylglyoxal (MGO) but are known to differ in their amounts of other metabolites. Retail honey samples labelled as 'mānuka', manuka, or 'tea tree' honey sourced from New Zealand (n = 34) and Australia (n = 35), were measured using laser assisted-rapid evaporative ionisation mass spectrometry (REIMS) in positive and negative ionisation modes, with 1637 and 1744 molecular features detected, respectively. Country of origin was strongly reflected in the REIMS fingerprints irrespective of similarity of methylglyoxal, with >50% of detected features differing between New Zealand and Australian-sourced honey. Combined REIMS features in negative ionisation mode correlated strongly with current molecular markers of mānuka honey quality ($r^2 > 0.9$). *Leptospermum* honey from New Zealand and Australia have distinct molecular fingerprints, potentially due to evolutionary and genomic differences between the predominant *Leptospermum* species, bees, environment and honey processing.

1. Introduction

Mānuka honey is highly sought after and one of the most valuable kinds of honey produced worldwide (Hegazi, Elghani, & Farag, 2022), yet there is a dispute about what constitutes mānuka honey, and in particular if it can only come from bees feeding on the nectar of the mānuka tree (*Leptospermum scoparium*) in New Zealand, or if it extends to the same species of tree in Australia or honey from the broader *Leptospermum* genus. Around 83 species of *Leptospermum* trees are widespread in Australia, while in New Zealand, only *L. scoparium* is widespread (Dawson, 2009), with ancestors of the current tree thought to have spread to New Zealand from Australia during the Miocene period (5–23 million years ago) (Thompson, 1989). The word mānuka is the Māori (indigenous people of New Zealand) word for *Leptospermum scoparium*, and the tree has a long history of use in traditional healing. However, honey from the nectar was not produced until European colonisers introduced the honeybee (*Apis mellifera*) to New Zealand in 1839. Interest in mānuka honey from New Zealand gained ground in the 1990s when scientific studies reported that it had unique antibacterial properties and improved wound healing (Willix, Molan, & Harfoot, 1992). The non-peroxide antibacterial activity was attributed to methylglyoxal (MGO). During honey production in the hive, MGO is non-enzymatically converted from dihydroxyacetone (DHA), which is found in the nectar of mānuka flowers (Adams, Manley-Harris, & Molan, 2009; Williams et al., 2014). The public awareness of the antibacterial activity, and its representation by the amount of MGO, has increased demand for mānuka honey highly, and it is consequently worth double or more of high-grade honey from other nectar sources (Hegazi et al., 2022). MGO has subsequently been found to be present in some Australian *Leptospermum* spp., honey (Cokcetin et al., 2016), leading to Australian honey producers re-naming honey from *Leptospermum* spp., nectar, including 'jellybush' honey, as 'manuka' honey. New Zealand's Ministry for Primary Industries (MPI) developed a definition for mānuka honey based on *Leptospermum scoparium* pollen DNA and the combination of metabolites 2′-methoxyacetophenone (2′-MAP), 2-methoxybenzoic acid (2-MBA), 4-hydroxyphenyllactic acid (4-HPLA) and 3-phenyllactic acid (3-PLA) that is unique to mānuka honey (McDonald, Keeling, Brewer, & Hathaway, 2018; Rückriemen & Henle, 2018). In a small-scale study, 2′-MAP was below instrumental limits of detection in Australian *Leptospermum* honeys, while the other markers were present in different concentrations to New Zealand *L. scoparium* honey, suggesting that the MPI molecular definition for mānuka honey

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verification could also determine geographic origin (Rückriemen & Henle, 2018). We note that the correct spelling of the common name of *L. scoparium* is mānuka, as this comes from the Māori language, rather than 'manuka'. Throughout this paper we have used mānuka, unless specifically referring to labels that state 'manuka'. The 'ā' is a long 'a' sound, placing the emphasis on the first sylliable.

Against the background of whether mānuka honey should be defined by chemistry or geographic origin is potential for fraud in mānuka honey (Hegazi et al., 2022). Fraudulent methods include adulteration by syrup or cheaper honey to mimic the brown colour of mānuka honey, mislabelling multifloral mānuka honey as monofloral mānuka honey, and the addition of the chemicals MGO and DHA to honey or coloured syrup. There is also potential for the chemicals that are used for the MPI definition to be added to syrup or non-mānuka honey. There are many methods used to test for honey fraud, but given the skill applied to make fraudulent honey, these methods need to continually evolve to be hard to cheat.

Several publications report the use of metabolomics to test for honey fraud, with promising results (Schievano, Finotello, Uddin, Mammi, & Piana, 2016; Spiteri et al., 2015). Metabolomics is attractive as it measures hundreds of small molecules in a single sample and characterises the whole sample rather than just a few key components present. However, nuclear magnetic resonance spectroscopy and chromatography-mass spectrometry-based methods commonly used for metabolomics require sample extraction or, at a minimum, dilution before analysis, thus reducing throughput. A relatively new development in mass spectrometry for food analysis is rapid evaporative ionisation mass spectrometry (REIMS), which has shown potential for fast honey analysis (Wang, Cao, Han, Pei, Ren, & Stead, 2019). A sample is vapourised, the resulting aerosol is drawn through and ionised by the REIMS source, and a fingerprint of the metabolites present in the aerosol is detected by the mass spectrometer within a few seconds of the vaporisation process. REIMS was initially developed for real-time detection of tumours during cancer surgery (Balog et al., 2013) and has been proven to be a versatile direct analysis mass spectrometry method for food as well, with scientific studies using REIMS to study meat (Ross et al., 2021), fish and other seafood (Black et al., 2017), and fermented dairy products (Murphy et al., 2021). The output from a REIMS analysis is a high-resolution mass spectrum, usually from m/z 50–1200, also referred to as a mass spectral 'fingerprint', and can be used to determine whether samples differ based on their small molecule composition. Only one published study has previously reported the use of REIMS to measure honey, finding that REIMS was able to successfully determine the difference between honey of different botanical origins, rice and corn syrup, and dilutions of honey with rice or corn syrup (Wang et al., 2019). That study used an electronic knife to vaporise samples for REIMS analysis, and use of a laser has been demonstrated as an alternative method for sample vaporisation prior to REIMS detection (Genangeli, Heeren, & Porta Siegel, 2019). 'Laser-assisted'(LA)-REIMS has an advantage of improving consistency as sampling time can be precisely controlled and inter- and intra-user variation is removed in contrast to the more widely used electronic knife. To date, no studies have reported the use of LA-REIMS for honey analysis.

We hypothesised that country of origin of mānuka-labelled honey would be detectable using REIMS. We also hypothesised that features within the REIMS fingerprints would be associated with other measures of honey quality, including the MPI measures of mānuka honey. To test this hypothesis, we measured 67 honey samples from New Zealand (all from *L. scoparium*) labelled as 'mānuka', and Australia (from *Leptospermum* spp) labelled as 'manuka', 'mānuka' or 'tea tree' honey, using laser-assisted REIMS. The resulting fingerprints were analysed to determine if the country of origin was detectable in the fingerprints and whether features in the fingerprints were correlated with quality measures commonly applied to mānuka honey.

2. Materials and methods

2.1. Honey samples

Honey samples were a convenience sampling of honey available via eCommerce platforms in Australia ($n = 35$), and New Zealand honey ($n = 34$) matched for the measured MGO concentrations in the Australian honeys (Supplemental Table 1). These honey samples represent a large proportion of the commercially available 'manuka' honey in New Zealand and Australian markets. They can be seen to be broadly representative of what is available to consumers. The Australian honey samples were sourced from companies based in Western Australia, Queensland, New South Wales, Victoria and Tasmania. The samples had wide variation in colour, from dark caramel to black, and viscosity. One honey label on the jar specified the *Leptospermum* species as a mixture of *L. scoparium* and *L. lanigerum*, while no *Leptospermum* species were noted on all the remaining samples. The New Zealand honeys obtained from suppliers from different regions of the North and South Islands of the country were assumed to come from *L. scoparium* as this is the only *Leptospermum* spp. widespread in New Zealand. Honey samples were stored at −20 °C before analysis. The 33 of the 34 New Zealand honey samples met the MPI chemical definition of monofloral mānuka honey, with the remaining sample meeting the criteria for multifloral mānuka honey. Two of the Australian sourced honeys were removed from the country of origin analysis due to uncertainty of botanical origin from the source labelling but were retained for correlation analysis. This results in $n = 33$ for Australian honey in the country of origin analysis and $n = 35$ for Australian honey for other analyses. Honey samples were imported into New Zealand under a transitional facility permit issued by the New Zealand Ministry of Primary Industries and analyses carried out in approved transitional facilities. A honey sample with an MGO rating of '10+' and independent of the study samples was used as a quality control sample.

2.2. Honey analyses

Honey samples were analysed for dihydroxyacetone (DHA), methylglyoxal (MGO), and hydroxymethylfurfural (HMF) using a reduced run-time modification of the HPLC method of Windsor, Pappalardo, Brooks, Williams, and Manley-Harris (2012), leptosperin by HPLC-fluorescence, and 4-hydroxyphenylacetic acid (4-HPLA), 2-methoxybenzoic acid (2-MBA), 2′-methoxyacetophenone (2′-MAP), 3-phenyl lactic acid (3-PLA), and DNA, according to the MPI protocols (MPI, 2017b, 2017c), by an ISO 17,025-accredited laboratory (Analytica Laboratories Ltd, Hamilton New Zealand).

2.3. Classification of honey samples using the MPI markers

Honey samples were characterised using the MPI science definition for mānuka honey (MPI, 2017a), where monofloral mānuka honey is defined as having 3-PLA ≥400 mg/kg and 2′-MAP ≥5 mg/kg and 2-MBA ≥1 mg/kg and 4-HPLA ≥1 mg/kg. Multifloral mānuka honey (honey coming from bees feeding on the nectar of mānuka and other flowers) is defined as having 3-PLA 20–399 mg/kg and 2′-MAP ≥1 mg/kg and 2′-MBA ≥1 mg/kg and 4-HPLA ≥1 mg/kg.

2.4. Rapid evaporative ionisation mass spectrometry analysis of honey samples

Honey samples were heated to 50 °C for 30 min and 2 mL pipetted into 12-well culture plates for analysis in randomised order using the random number function in Excel. Laser-assisted-rapid evaporative ionisation mass spectrometry (LA-REIMS) was used to measure the metabolite fingerprint of the honey samples. Samples were volatilised using a 60 W CO_2 laser mounted on a X/Y gantry robot (Thunder Laser Nova51, ITS Ltd, Rangiora, New Zealand) at 21% power, with a tube

transferring the resulting sample aerosol into the REIMS source. Burns were straight lines, 16 mm in length, with a laser speed of 4 mm/second and delay of 10 s between each sample. The flow rate for LC-MS grade isopropanol infusion into the REIMS source was 200 μL/min. The REIMS source was fitted to a Xevo G2 XS quadrupole time-of-flight mass spectrometer (Waters Ltd, Wilmslow, UK). The samples were analysed first in negative ionisation mode in time-of-flight mode only (i.e. no collision energy applied), scanning between *m/z* 50–1200 with a scan rate of 2 Hz, followed by positive ionisation mode, using the same settings. The REIMS instrument was mass-calibrated before analyses using sodium formate.

Prior to starting this work, we optimised the laser settings, based on applying sufficient power to generate a vapour to reach at least 1e7 total intensity in negative mode. Power was first stepped in 2% increments from 10% power, followed by 1% increments. We determined that 21% power was the ideal power setting for Leptospermum honey with our instrumentation.

2.5. Data processing and statistical analysis

Data for each plate were acquired using MassLynx (Waters), and data for individual samples were isolated using ProGenesis Bridge (Waters), which also performed a mass alignment. Spectra were aligned to the sucrose/disaccharide mass for both ionisation modes (*m/z* 377.0856 for negative mode $(M+Cl]^-$, and *m/z* 365.1054 for positive mode ($[M + Na]^+$). Data were further processed using ProGenesis QI software (Waters) to detect and align features and adducts across all the samples and normalise individual peak areas based on overall peak intensity. Automated peak picking was used to detect REIMS features, using a threshold value of 4. REIMS data were also matched against the Human Metabolome Database (Wishart et al., 2021) and LipidMaps database (Sud et al., 2007) for possible identifications based on high-resolution mass, with a maximum mass error of 5 ppm. Due to the often tentative nature of these database matches and without corroborating mass fragmentation spectra, we have not extensively used this data beyond assessing whether there is consistency in tentative matches belonging to chemical structure classes or subclasses. When reviewing database matches, those that were highly unlikely to be found in nature, such as pharmaceutical compounds, were considered to not have any match, as were masses where there was a high degree of heterogeneity in potential compound class. The high-resolution mass of the honey markers measured was estimated using Insight Explore software (Shimadzu, Kyoto, Japan).

Data were exported for further processing in Excel, where features in the quality control sample with >30% variation were removed to remove likely noise in the REIMS spectra. Features that were removed in this process were generally of low abundance (<200 ion intensity units). Data tables with available sample information, including DHA, MGO, HMF, 4-HPLA, 2-MBA, 2′-MAP, 3-PLA, and leptosperin measurements, were constructed and imported into SIMCA multivariate data analysis software (Satorius, Umeå, Sweden). REIMS data were set as *X*-variables, and continuous honey analysis data (DHA, MGO etc.,) were set as *Y*-variables. Data were scaled to unit variance before multivariate data analysis.

Data were initially inspected using principal components analysis (PCA) to determine the main sources of variation in the data. Orthogonal projection to latent structures-discriminant analysis (OPLS-DA) was used to identify if the country of origin could be modelled from the data and, if so, what metabolite features best defined this difference. OPLS-DA modelling is interpreted using 'R2X' (the amount of variation between groups that can be explained by the X variables in the model – in this case REIMS features), and 'Q2' (the reproducibility of the model based on cross-validation of the data, where in the context of biological samples 0.9–1 is very good, 0.5–0.9 is good, 0.2 moderate and <0.2 is poor). The difference between geographic origin was further tested by building a feature-reduced model removing features with a variable importance projection (VIP) <1.5 and then remodelling the data using the unsupervised PCA method (Worley & Powers, 2016). We used these 'feature reduced' PCA scores plots to visualise data as they provide a more accurate representation of data variance after variable selection than OPLS-DA scores plots, which can be misleading with regards to differences between groups. Data normality was tested using Sharpiro-Wilk tests, and data were found to have variable distributions depending on the REIMS feature. Thus non-parametric tests were used for determining further differences. Differences for individual features were determined using Mann–Whitney tests, with multiple testing corrected using the Benjamini-Hochberg false discovery rate (Benjamini & Hochberg, 1995). Differences were considered significant if false discovery rate p was <0.05. Data analysis for positive and negative ionisation modes were carried out separately. The ability to correctly classify honey samples as coming from New Zealand or Australia was tested using misclassification analysis and receiver operating characteristic curves, using analyses built into SIMCA. Data were further classified based on their *L. scoparium* pollen DNA measurement by qPCR. New Zealand and Australian honey with a value of <36 Cq for *L. scoparium* pollen DNA were further compared to determine if the samples could still be separated based on country of origin. Cq refers to the number of cycles of the qPCR instrument before detection of the appropriate genetic material sequence, with a maximum of 40 cycles. The amount of genetic material is inversely proportional to the Cq value.

Although not designed to investigate differences between regions within the two countries studied, we have explored whether regional differences could be detected based on the REIMS fingerprints. Australian states were used as regional classifiers, acknowledging the heterogeneity of landscapes within each state. There were insufficient samples from Western Australia to include this state in the analysis. The New Zealand regional collections were from across the country with insufficient samples from individual provinces to allow a comparison province by province. Instead, honey samples were grouped based on whether they were from the South Island, Lower North Island, or Upper North Island. Again, within these broad regional classifications there is substantial heterogeneity in landscapes, which limits the interpretation to 'proof of principle' based on the data collected.

Relationships between the REIMS data and honey measurements were explored using partial least squares (PLS), a method for multivariate correlation. Data were log transformed prior to correlation analyses as some features were not normally distributed. Each individual honey measurement was explored separately for individual REIMS features that correlated with that measurement. PLS-based predicted versus measured values for each honey measurements were also determined using SIMCA to explore whether the use of all REIMS variables could outperform individual features.

Composite spectra using the average mass intensity for New Zealand and Australian honeys were generated using Abstract Model Builder v 1.0.2153.0 software (Waters) to bin the spectra (3*m/z*) units and Excel (Microsoft) to visualise the spectra).

3. Results and discussion

3.1. Classification of honey samples using the MPI definition and other markers of honey quality

Three of the 35 Australian honey samples met the multifloral mānuka honey criteria, while the rest were classified as non-mānuka honey, according to the MPI mānuka honey definition. Of the 34 New Zealand honeys, 33 met the definition for monofloral mānuka-honey, while one was classified as multifloral-mānuka honey.

Of the honey markers measured in this study, only MGO and leptosperin did not differ based on country of origin (Supplemental Table 1), with similar MGO values being one of the criteria for selecting the New Zealand honey samples used in this study. Of the MPI markers used, average values differed between countries, reflecting that none of the Australian honeys met the chemical definition for mānuka honey set

by MPI. It was also notable that HMF was higher in Australian honeys compared to New Zealand honey, suggesting that either the Australian honeys were more likely to have been thermally treated or kept for longer – both treatments which elevate HMF.

3.2. Detection of honey features using LA-REIMS

LA-REIMS analysis of honey generated a good signal for the REIMS. To our knowledge, this is the first report of a laser being used to generate the aerosol for honey analysis. REIMS spectra in both positive and negative ionisation modes were dominated by features detected at $< m/z$ 500, though several larger masses were detected in negative ionisation mode (Fig. 1). Visually, the spectra differ from those previously reported for acacia, canola, chaste, jujube, citrus, and medlar honey acquired using an electronic knife in negative mode (Wang et al., 2019). However, in meat it has been reported that use of a laser or electronic knife to generate an aerosol for REIMS can lead to a difference in the ratio of ions in the resulting mass spectrum (Genangeli et al., 2019), suggesting that spectra generated using an electronic knife or laser should not be expected to be similar. A total of 1637 features were detected in positive ionisation mode, and 1730 features were detected in negative ionisation mode after removing features that had QC variation >30%. At a practical level, the use of a laser interface was more convenient due to better standardisation of measurement time and no requirement to use a conductive holder for the honey samples that is a requirement for the electronic knife.

3.3. Differences based on geographic origin

3.3.1. Comparison of country of origin of all 'mānuka' or 'manuka' labelled honey samples

The differences between the New Zealand and Australian honey samples were substantial, with 61% (1058) of the features detected in negative ionisation mode and 57% (930) of those detected in positive mode differing at $p_{adj} < 0.05$. Based on these samples, the metabolite composition of mānuka-labelled honey from New Zealand and Australia are substantially different from each other. The data processing method accounts for adducts of the same molecule. However, there is a chance that some adducts of the same metabolite have not been grouped together, inflating the number of features detected. Principal components analysis modelling found that for negative mode REIMS analysis, the country of origin was separated along PC1 (Fig. 2), explaining 25% of overall variation. In contrast, for positive mode REIMS analysis, there was some overlap along PC1, with PC3 (7%) and PC8 (3%) also showing a general difference between honey from New Zealand and Australia. The use of supervised analysis (OPLS-DA) to focus on the features that best predicted the difference between New Zealand- and Australian-sourced honey resulted in strong models based on all data (Table 1). PCA models using features from OPLS-DA modelling that best-predicted country of origin resulted in the separation of honey samples from New Zealand and Australia along the first component (Fig. 2), explaining 60 and 69% of the variation in negative and positive mode, respectively. Features that best explained the difference between countries were spread across the mass range of REIMS spectrum (Tables 2 and 3). Consistency of differentiation between the two collection periods for this study (2020 and 2021) found that country was readily differentiated for both years (Table 1), even though statistical power was substantially reduced.

Regional variation within the two countries was also explored, as several studies have reported chemical variation in mānuka honey based on region. For neighbouring regions, the crude OPLS-DA models were often poor (e.g. Queensland vs New South Wales, and New South Wales vs. Victoria), but improved on feature reduction. The estimates of false and true positive rates using ROC AUC values were also more likely to be below 1 for neighbouring regions, though misclassification tables found that the models were able to correctly classify most honey samples.

3.3.2. Monofloral and multifloral mānuka honey

Applying the MPI definition based on chemical markers without the additional DNA test to the honey samples collected for this study found that 33 of the New Zealand honey samples were classed as 'monofloral' mānuka honey, and one classified as 'multifloral'. None of the Australian honey samples met the criteria for 'monofloral' mānuka honey, though two were classified as 'multifloral' mānuka honey. Given the low number of honey samples within the 'multifloral' category, we did not compare the country of origin for multifloral samples.

3.3.3. Comparison of the country of origin of honey containing *L. scoparium* pollen DNA

An additional component of the MPI chemical definition of mānuka honey is whether it contains DNA from *L. scoparium* pollen. Of the honey samples analysed, 29 New Zealand samples and seven Australian samples were below the required threshold of Cq 36 for being considered to contain mānuka pollen. The DNA results may have been impacted by the age of the honey samples, with Australian honey samples having higher concentrations of HMF, a marker positively associated with honey age, compared to New Zealand samples. DNA from pollen in honey is known to degrade with age (personal communication to T Braggins).

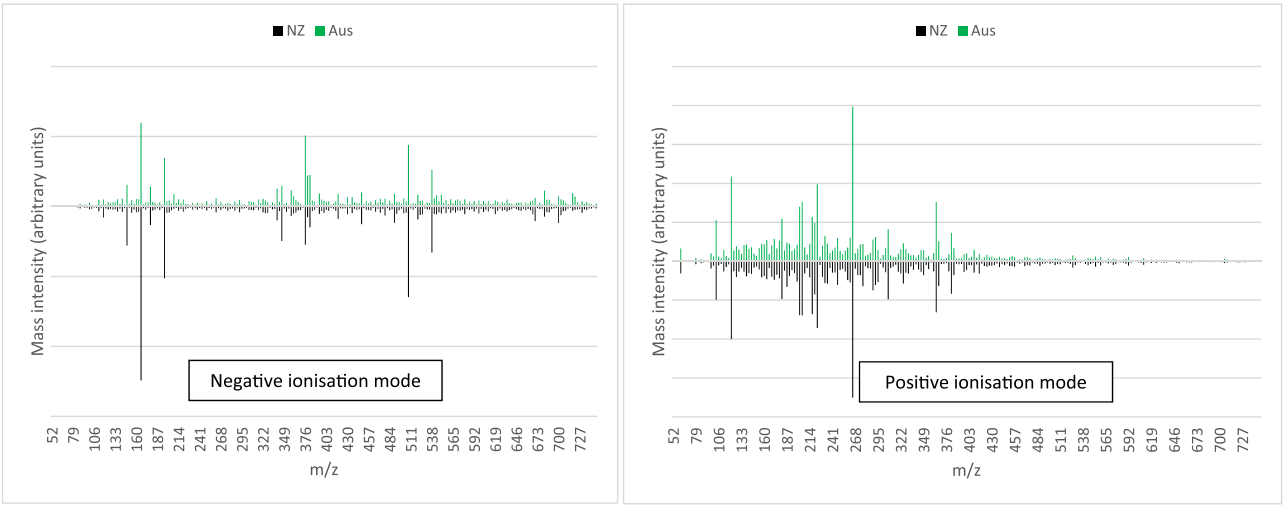

Fig. 1. Composite REIMS positive and negative ionisation mode spectra for New Zealand and Australian *Leptospermum* honeys. Spectra have been binned to a width of 3 m/z units from m/z 50–700 and average mass intensity across all samples used to build the spectra.

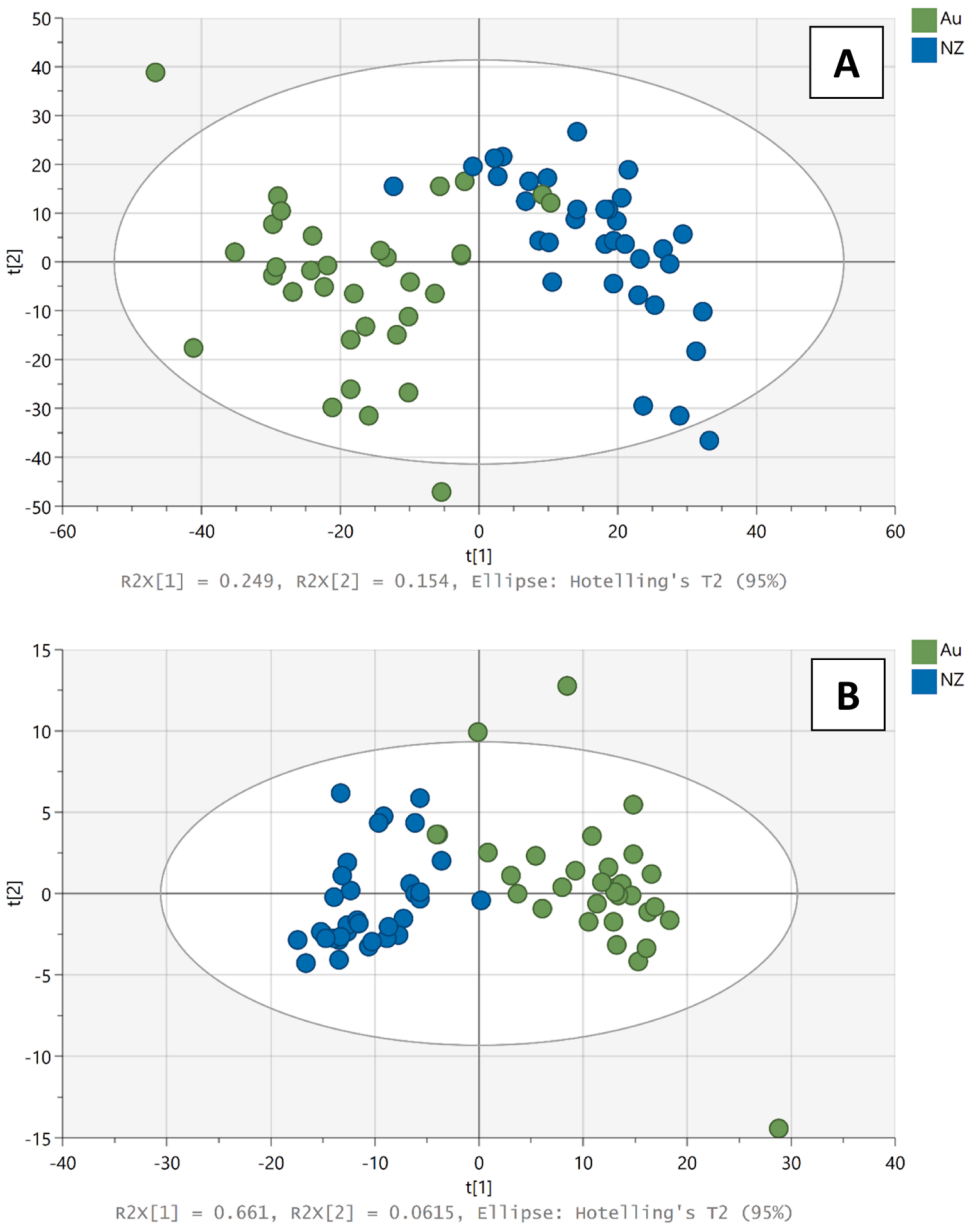

Fig. 2. PCA scores plots of REIMS data from negative mode and positive mode analyses, modelled with the full fingerprint and with a number of features included reduced using a threshold of VIP>1.5 based on OPLS-DA modelling. A; negative mode, full dataset. B; negative mode, feature reduced dataset. C; positive mode, full dataset. D; positive mode, feature reduced dataset.

Comparing the REIMS fingerprints of these honey samples found that there was still a clear difference between New Zealand and Australian honey samples based on PCA analysis of negative-mode REIMS data (Fig. S1) and OPLS-DA modelling (R2X = 0.77, Q2 = 0.71). In univariate analysis for the honey samples containing *L. scoparium* pollen at Cq < 36, 872 features differed at $P_{adj} < 0.05$ between New Zealand and Australian honey. For REIMS analyses in positive ionisation mode, results were similar (Fig. S1), with an OPLS-DA model from the full fingerprint of (R2X = 0.54 and Q2 = 0.44) and 905 features differing at $P_{adj} < 0.05$. These results suggest that the chemical fingerprint of honey differs between the two countries, even in samples that contain DNA from pollen matching that of *L. scoparium*.

3.3.4. Can the features detected by REIMS that differentiate the country of origin be identified?

The statistical analyses suggest that there is a strong signature in honey related to geographic origin. Identification of these features is limited to database matching using high-resolution mass, equivalent to level 3 metabolite identification confidence (putatively annotated compound class) (Salek, Steinbeck, Viant, Goodacre, & Dunn, 2013). Of the 40 features in each ionisation mode with the largest fold change difference and $P_{adj} < 0.01$ between New Zealand and Australia, 16 features had plausible tentative compound class identifications in negative mode and nine in positive mode. REIMS analysis is not typically used for identification as automated mass fragmentation is not currently possible. However the high resolution masses do give some indications of what compound classes differ between New Zealand and Australian *Leptospermum* honeys (Tables 2 and 3). Among the possible identifications that were higher in Australian honey were saccharides (pentose monosaccharide; *m/z* 133.0508 $[M\text{-}H_2O\text{-}H]^-$, disaccharide; *m/z* 377.0858 $[M + Cl]^-$, dipentose; *m/z* 317.0642 $[M + Cl]^-$) and phenolic compounds including a possible anthocyanin (*m/z* 722.2086

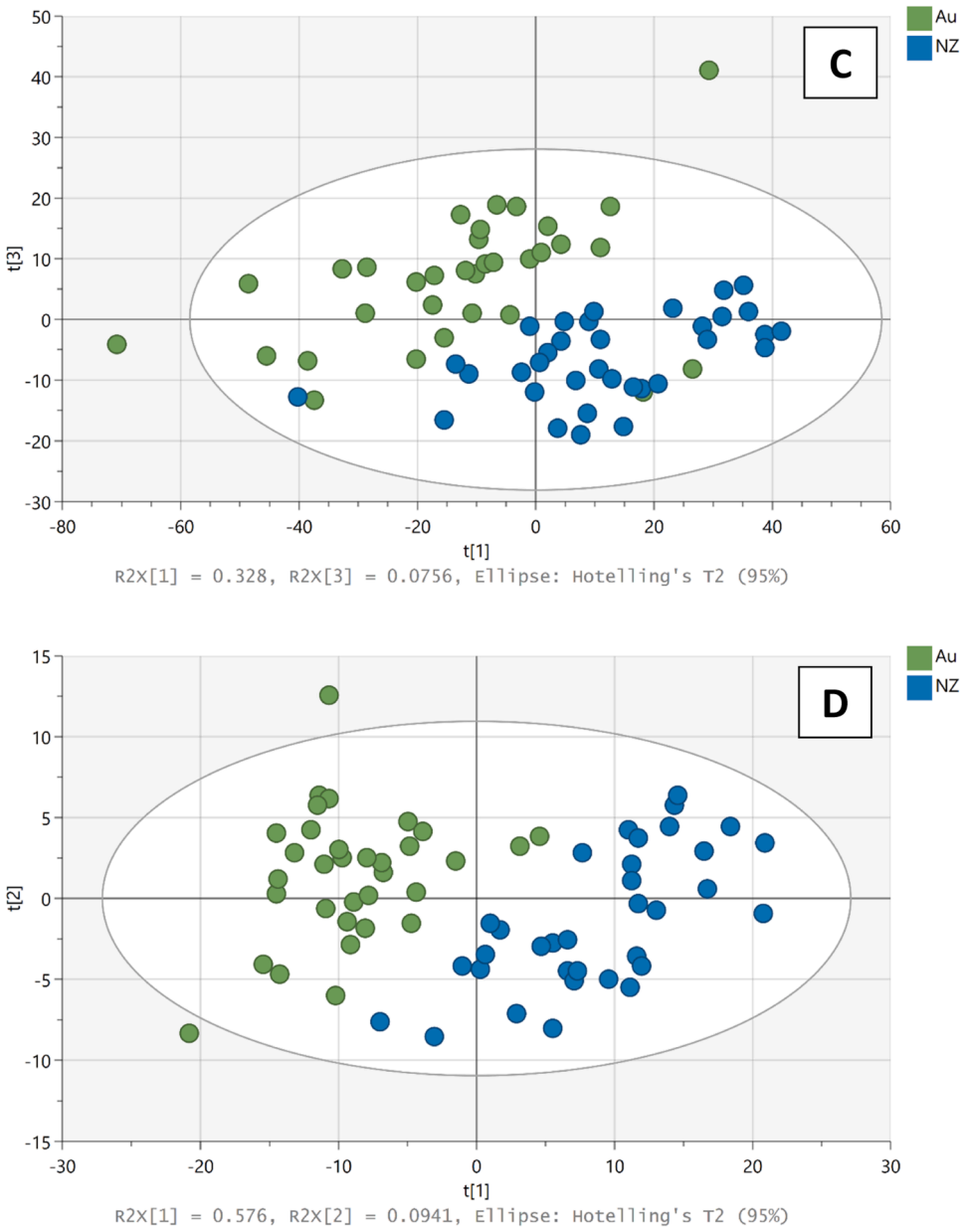

Fig. 2. (*continued*).

[M-H_2O-H]$^-$) and glycosylated flavonoids (*m/z* 721.2017 [M-H_2O-H]$^-$, *m/z* 883.2504 [M-H_2O-H]$^-$). In positive mode, *m/z* 135.0439 [M-H_2O+H]$^+$ and *m/z* 153.0546 [M + H]$^+$ were tentatively identified as phenolic acids or alkylphenylketones, and *m/z* 437.1251 [M + H]$^+$, *m/z* 707.2205 [M-H_2O+H]$^+$, *m/z* 709.1982 [M-H_2O+H]$^+$ and 731.1781 [M + Na]$^+$ were tentatively identified as flavonoids and glycosylated flavonoids. The higher intensity of the disaccharide mass could suggest an overall higher sugar concentration, though this was not measured in the present samples. We explored whether this alone could account for the difference between New Zealand and Australian honey by dividing all features in each sample by its *m/z* 377.0858 intensity. There was no reduction in the ability to distinguish between the two countries based on the *m/z* 377.0858 corrected fingerprint. This suggests that while this feature differed between honey from the two countries, it was not the only defining feature.

Of the tentatively identified compounds higher in New Zealand honeys, one was tentatively identified as phenyllactic acid (*m/z* 165.056 [M-H]$^-$), which is included in the MPI panel for identification of mānuka honey. This same mass correlated strongly with LC-MS/MS measurement of 3-PLA ($r = 0.95$) (Supplemental Table 2). In positive mode, features that were higher in New Zealand honeys with tentative identification included two dipeptides (*m/z* 174.1123 [M + H]$^+$, *m/z* 294.1534 [M + Na]$^+$) and leptosperin (*m/z* 575.1350 [M + K]$^+$.

Due to the high number of REIMS features that differed between New Zealand and Australian honey and the tentative nature of the high-resolution mass identifications, we did not explore potential identifications of all features and note that use of complementary methods could be used in the future to further explore the nature of metabolites that differ between New Zealand and Australian honey samples.

3.4. Basis for geographic origin leading to difference in honey metabolite composition

The clear metabolite composition difference between honey from New Zealand and Australia is likely to be based on a combination of genetic factors (Koot et al., 2022) in relation to the *Leptospermum* spp.

Table 1
OPLS-DA model characteristics for crude and feature reduced models for the comparison between New Zealand and Australian honeys collected in 2020 and 2021 together and for each individual year. Crude model includes all variables, while the feature reduced model is based on all features with a VIP>1.5 in the crude model, broadly selecting those features that explain difference between geographic origins. ROC-AUC: Receiver operating characteristic, area under the curve. NZ: New Zealand, Au: Australia. R2X: amount of variation between groups explained by the REIMS data. Q2: OPLS-DA model validation statistic reflecting the reproducibility of the model. For a reproducible model, R2X and Q2 should be numerically close.

	Crude model (all features)		Feature reduced model		ROC-AUC featured reduced model	Misclassification table correct%
Negative mode						
	R2X	Q2	R2X	Q2		
All honey (*n* = NZ: 34, Au: 33)	0.79	0.75	0.81	0.80	NZ: 1 Au: 1	100%
Honey purchased in 2020 (*n* = NZ: 9, Au: 10)	0.78	0.62	0.83	0.80	NZ: 0.97 Au: 1	100%
Honey purchased in 2021 (*n* = NZ: 25, Au: 23)	0.84	0.82	0.86	0.85	NZ: 0.97 Au: 1	100%
Positive mode						
	R2X	Q2	R2X	Q2		
All honey (*n* = NZ: 34, Au: 33)	0.48	0.44	0.70	0.69	NZ: 1 Au: 1	100%
Honey purchased in 2020 (*n* = NZ: 9, Au: 10)	0.58	0.45	0.80	0.76	NZ: 0.95 Au: 0.95	100%
Honey purchased in 2021 (*n* = NZ: 25, Au: 23)	0.57	0.52	0.74	0.73	NZ: 1 Au: 1	100%

that produce the nectar, environmental influences such as climate and soil (Santos, Hancox, Picanço, Delaporte, & Hogendoorn, 2023), differences in both bees and beekeeping, and how the honey is processed and stored. Disentangling these factors is difficult, though several available chemical markers for both honey quality and mānuka honey can be informative. One contributing factor to the difference between New Zealand and Australian *Leptospermum* honey, including those from *L. scoparium*, is that *L. scoparium* has likely been separated between the countries for at least 5 million years (Thompson, 1989). There are suggestions that trees identified as *L. scoparium* in Tasmania are potentially a different species and, indeed, a different variety (Bond, Dickinson, & Mark, 2004). This would support that a genetic and environmental component may lead to differences in nectar.

The tentative identification of the REIMS features that strongly differed between New Zealand and Australian honey were likely to be plant-secondary metabolites, suggesting a relationship with *Leptospermum* nectar composition. The DHA and mānuka honey authenticity markers such as leptosperin and 3-PLA are associated with flower development, and interaction between *L. scoparium* cultivar and soil composition influenced nectar yield, though not DHA (Nickless, Anderson, Hamilton, Stephens, & Wargent, 2017). This possibly explains that while DHA and MGO might be present in many *Leptospermum* spp-derived honey, they can still vary in their broader composition. Other studies have also found that a combination of environment and genotype influences *L. scoparium* nectar composition (Clearwater, Revell, Noe, & Manley-Harris, 2018), while nectary photosynthesis also influences DHA (Clearwater et al., 2021). Few studies have compared the composition of *Leptospermum* spp nectar. However, the ratio of sugar to DHA was found be high in Australian *Leptospermum* spp compared to New Zealand *L. scoparium* (Williams, Pappalardo, Bishop, & Brooks, 2018). Other *Leptospermum* species in Australia contained no detectable DHA in their nectar (Williams et al., 2018). DHA/total sugars were also found to vary widely based on region, year and cultivar (Williams et al., 2014). Notably, within-site variation for DHA/total sugars has been reported as being more significant than between-site variation for *L. scoparium* in New Zealand (Noe, Manley-Harris, & Clearwater, 2019), suggesting that there is some consistency in the production of DHA across New Zealand *L. scoparium*. In this study, the honey samples were selected to be similar in their MGO content ($p = 0.52$), so variation of DHA in nectar may not necessarily explain the observed variance between honey samples related to geographic origin.

Several phenolic acids are detected in mānuka honey (Stephens et al., 2010; Yao, Datta, Tomás-Barberán, Ferreres, Martos, & Singanusong, 2003), and some of these are used as part of the mānuka honey authentication panel developed by New Zealand's MPI (Rückriemen & Henle, 2018). The phenolic acid content was found to increase with age and heat treatment (Stephens et al., 2010), factors which are unknown in the samples used in this study. Based on HMF measurements, Australian honeys may have been older or exposed to greater heat, which on one hand may increase the phenolic acid content, but did not lead to higher amounts of the phenolics that are part of the MPI mānuka honey definition. Phenolic compounds kojic acid, acetyl-2-hydroxy-4-(2-methyoxyphenyl)-4-oxobutanate, and 3-hydroxy-1-(2-methoxyphenyl)-penta-1,4,-dione were found to be higher in mānuka honey compared to both kānuka and a single jellybush honey sample using LC-MS (Beitlich, Koelling-Speer, Oelschlaegel, & Speer, 2014). At the same time, 2′-MAP and 2-methylbenzofuran were higher in mānuka honey using headspace GC–MS analysis (Beitlich et al., 2014). The same study found that linalool oxide and 3,4,5-trimethylphenol were higher in the jellybush honey sample compared to the New Zealand honeys (Beitlich et al., 2014). Phenolic flavonoids quercetin, isorhamnetin, chrysin and luteolin, and an unknown flavonoid, have been reported to be the main flavonoids in mānuka honey, in contrast to myricetin, luteolin and tricetin being the main flavonoids in jellybush (*Leptospermum polygalifolium*) honey (Yao et al., 2003). Based on the tentative identifications in the present study, the difference in the MPI definition phenolics measured, and these earlier studies, plant-secondary metabolites, including phenolic compounds, may be critical distinguishing features between *Leptospermum* honeys from different geographic origins.

3.5. Metabolomics and mānuka honey origin

To date, few studies have used metabolomics to study honey's composition and relate this to geographic origin. One study using LC-MS and NMR metabolomics to compare honey from Malaysia and New Zealand, including some mānuka honey samples, finding a diverse range of low-molecular-weight compounds present in honey, with up to 2720 and 2079 features detected using LC-MS in positive and negative modes, respectively (Yusoff, Abbott, Young, & Edrada-Ebel, 2022). Data processing settings can have a strong influence on these numbers, but does underline the chemical diversity of honey metabolites and notably is in the same range as what has been detected using REIMS in the present study. The comparison between New Zealand and Malaysian honey grouped together honey from various sources, including manuka and multifloral honey blends. There was a clear separation between Malaysian and New Zealand honey and between honey types collected in the North and South islands of New Zealand (Yusoff et al., 2022), again supporting that geography influences honey composition, even if the nectar comes from the same species of plant. In this case the authors found that acetylated glycosides were higher in North Island mānuka

Table 2
The 40 most important negative and positive ionisation REIMS features for discriminating between New Zealand and Australian manuka labelled honey, based on greatest fold change, FDR corrected *p*-value <0.05, and >0.05% of overall spectral intensity. Data are ion counts for each *m/z* value. Data are median and interquartile range, and *p*-values are Benjamini–Hochberg false discovery rate corrected Mann–Whitney *p*-values.

Feature *m/z*	Australian honey	New Zealand honey	Fold change	P_{corr}	Possible ID
Negative mode					
Metabolite features higher in Australian honey					
209.0814	6364 ± 6249	264 ± 137	0.04	2.24E−15	Dihydroxyphenolic acid
133.0508	1165 ± 1103	147 ± 38	0.13	1.32E−15	Pentose
389.1471	963 ± 959	149 ± 67	0.15	2.69E−09	Phenol or terpenoid glycoside
720.2053	5109 ± 2775	809 ± 560	0.16	1.61E−10	
551.1962	6322 ± 6533	1089 ± 326	0.17	1.68E−12	
719.2021	19,927 ± 9975	3798 ± 2428	0.19	5.42E−11	
721.2017	8131 ± 4250	1626 ± 876	0.2	7.21E−12	Flavonoid glycoside
1061.321	2797 ± 1336	623 ± 273	0.22	8.69E−10	
1062.327	1080 ± 550	249 ± 109	0.23	1.11E−08	
1063.323	1305 ± 630	313 ± 105	0.24	2.07E−11	
722.2086	1862 ± 1230	482 ± 328	0.26	4.22E−10	Anthocyanin
377.0858	64,089 ± 32,660	17,467 ± 9361	0.27	3.49E−11	Disaccharide (sucrose?)
379.0833	23,311 ± 11,204	6728 ± 3020	0.29	3.39E−11	
361.0738	5256 ± 2351	1699 ± 702	0.32	9.39E−13	Glycosylated pentose
1403.435	784 ± 454	256 ± 77	0.33	3.89E−10	
359.0755	12,727 ± 5991	4245 ± 2151	0.33	8.26E−12	Flavonoid
317.0642	5828 ± 2461	1978 ± 1020	0.34	1.57E−11	Dipentose
883.2504	1142 ± 744	396 ± 370	0.35	1.64E−06	Tetra-glycosylated flavonoid
437.1058	7865 ± 2935	2831 ± 1281	0.36	4.14E−12	Glycosylated phenolic acid
1079.331	932 ± 384	343 ± 132	0.37	5.44E−10	
Metabolite features higher in New Zealand honey					
702.2322	1632 ± 1080	5435 ± 2949	3.33	2.35E−10	
346.123	1587 ± 1358	5430 ± 1602	3.42	1.56E−13	
165.056	30,806 ± 27,457	105,455 ± 29,860	3.42	2.53E−13	Phenyllactic acid?
103.0552	283 ± 254	977 ± 254	3.46	1.49E−13	Methylated phenol
435.1491	1071 ± 1054	3790 ± 1599	3.54	1.03E−13	
345.1189	7843 ± 7438	28,942 ± 8568	3.69	1.12E−13	Glycosylated terpenoid
539.1845	640 ± 1509	2393 ± 2500	3.74	4.36E−05	Flavonoid
478.1631	394 ± 487	1531 ± 860	3.89	2.49E−08	
195.0655	9716 ± 12,601	42,990 ± 40,267	4.42	3.05E−07	Phenolic acid
465.1586	741 ± 863	3337 ± 1545	4.5	4.96E−10	
134.0374	174 ± 282	806 ± 768	4.63	1.99E−07	
347.1249	228 ± 226	1091 ± 500	4.78	7.3E−15	
376.1333	586 ± 759	3225 ± 2800	5.5	3.28E−08	
375.1282	2706 ± 4458	16,840 ± 15,625	6.22	2.66E−08	
507.4	145 ± 419	1438 ± 1586	9.9	1.19E−06	
165.1864	298 ± 855	4417 ± 1787	14.81	3.02E−09	
165.1214	75 ± 326	2767 ± 1607	37	1.17E−09	
195.208	0 ± 10	311 ± 1249	311.44	1.26E−06	
165.2877	0 ± 25	874 ± 1101	873.96	8.62E−11	
494.1718	1 ± 352	1442 ± 1448	1441.84	3.17E−06	

honey and low molecular weight phenolic metabolites were higher in South Island honey (Yusoff et al., 2022), though many of the New Zealand honey samples used in the Yusoff et al. study were blends and may come from a mixture of plants. Metabolomics studies on species and geographic variation of honey in China found that both honey from the nectar of different species and geographic location could be differentiated from LC-MS metabolomics profile (Li et al., 2017). Markers for geographic location were different for honey from different species. For example, the monoterpene geranial could be used to distinguish between the geographic region of lychee honeys but not acacia honey (Li et al., 2017). In an international survey of honey samples using LC-MS metabolomics, New Zealand and Australian honey differed both from each other and from all the 20 other countries included (Jandrić, Frew, Fernandez-Cedi, & Cannavan, 2017). However, the number of samples used was low (n<5/ country) and the type of honey differed widely between countries. Leptosperin was noted as one of the potential identifying metabolites for New Zealand honey, of which two were mānuka honeys. However, it was also detected in an Australian meadow honey sample, raising questions about its specificity (Jandrić et al., 2017).

3.6. Correlation of REIMS features with standard mānuka honey measures

A secondary objective of this work was to determine if features detected by REIMS were related to standard honey measures used to define mānuka honey quality, including the MPI panel. Currently, LC-MS is most commonly used for these measurements (Rückriemen & Henle, 2018), and fingerprinting methods such as REIMS may be helpful as a screening method. Because REIMS detects many features simultaneously, and several features may be correlated to one marker we have used both single mass correlations and multivariate correlations to investigate whether features detected using REIMS could be proxy markers. Large correlation coefficients (R^2) were found for measured values for honey markers compared to predicted values based on REIMS measurements. Correlations with leptosperin, 4-HPLA, 2-MBA, 2′-MAP and 3-PLA were especially strong (Table 4). Negative ionisation REIMS features resulted in $\underline{R}^2 > 0.9$, while correlations were modest-good for positive ionisation REIMS features (0.71–0.97) (Supplemental figure 2). A wide range of individual REIMS features also correlated with the honey markers, with several having correlations >0.9 (Supplemental Table 2), suggesting a direct relationship with the honey marker metabolite. Screening the ten largest correlations for each ionisation mode with the high-resolution mass suggested that *m/z* 571.1426,

Table 3
The most important positive ionisation REIMS features for discriminating between New Zealand and Australian manuka labelled honey, based on greatest fold change, FDR corrected *p*-value <0.05, and >0.05% of average spectral intensity in at least one of the country of origin groups. Data are ion counts for each *m/z* value.

Feature *m/z*	Australian honey	New Zealand honey	Fold change	P_{corr}	Possible ID
Positive mode					
Metabolite features higher in Australian honey					
135.044	4293 ± 4115	1514 ± 822	0.35	2.85E−07	Phenolic acid or alkylphenylketone
153.055	4493 ± 3408	2153 ± 532	0.48	3.65E−08	Phenolic acid or alkylphenylketone
276.078	1014 ± 384	570 ± 166	0.56	5.91E−11	
437.125	1519 ± 423	927 ± 262	0.61	1.09E−08	Flavonoid
264.184	1691 ± 815	1066 ± 454	0.63	1.05E−05	
707.22	2577 ± 919	1681 ± 664	0.65	1.2E−07	Glycosylated flavonoid
292.173	1067 ± 1324	699 ± 347	0.66	0.006561	
709.198	1080 ± 408	712 ± 289	0.66	9.44E−06	Glycosylated flavonoid
348.098	879 ± 339	580 ± 267	0.66	0.000369	
509.144	1151 ± 356	761 ± 282	0.66	5.17E−06	
365.204	1226 ± 287	823 ± 296	0.67	3.07E−09	
275.074	9701 ± 2794	6633 ± 1594	0.68	2.66E−09	
304.173	790 ± 749	543 ± 141	0.69	4.79E−06	
366.109	7707 ± 1810	5379 ± 1061	0.7	1.6E−12	
349.108	2068 ± 420	1444 ± 697	0.7	4.5E−07	
731.178	1468 ± 425	1035 ± 345	0.71	6.99E−06	Glycosylated flavonoid
430.223	839 ± 540	592 ± 149	0.71	0.003521	
257.014	971 ± 459	692 ± 239	0.71	0.015992	
365.299	2352 ± 473	1683 ± 394	0.72	2.14E−10	
472.236	1760 ± 1378	1270 ± 438	0.72	0.002774	
Metabolite features higher in New Zealand honey					
397.192	721 ± 306	1244 ± 570	1.73	2.64E−06	
264.143	1953 ± 1393	3386 ± 1448	1.73	7.99E−06	
382.169	969 ± 646	1683 ± 866	1.74	5.78E−07	
258.134	4984 ± 1512	8771 ± 2863	1.76	2.96E−08	
346.157	1074 ± 396	1912 ± 987	1.78	9.76E−08	
174.112	1756 ± 1177	3137 ± 1094	1.79	3.4E−06	Dipeptide
276.144	6632 ± 2194	11,891 ± 4571	1.79	5.63E−08	
294.153	4892 ± 2324	8972 ± 4399	1.83	4.84E−08	Dipeptide
354.175	2420 ± 1296	4557 ± 1859	1.88	2.61E−08	
652.249	562 ± 417	1058 ± 633	1.88	4.61E−05	
343.165	462 ± 245	929 ± 349	2.01	2.63E−07	
325.168	661 ± 371	1345 ± 869	2.03	6.51E−06	
366.174	5442 ± 2041	11,255 ± 5110	2.07	5.98E−09	
295.157	634 ± 268	1321 ± 733	2.08	4.16E−08	
330.158	3410 ± 979	7340 ± 2942	2.15	3.78E−09	
367.181	667 ± 272	1549 ± 1078	2.32	1.87E−09	
575.135	1610 ± 1208	4105 ± 1690	2.55	1.29E−05	Leptosperin
206.152	388 ± 178	1000 ± 1914	2.58	1E−05	
331.168	359 ± 132	1108 ± 854	3.09	2.04E−11	
576.139	266 ± 345	911 ± 540	3.42	3.29E−05	

575.1354 and 559.1625 may be the $[M + Cl]^-$, $[M + K]^+$ and $[M + Na]^+$ adducts respectively of leptosperin, a glycosylated phenolic acid found in *Leptospermum* spp honeys (Bong, Prijic, Braggins, Schlothauer, Stephens, & Loomes, 2017). For 2-MBA, *m/z* 151.0399 and *m/z* 153.0546 are likely the $[M\text{-}H]^-$ and $[M + H]^+$ adducts of 2-MBA,and *m/z* 165.056 is likely the $[M\text{-}H]^-$ adduct of 3-PLA. None of the other high-resolution mass differences between the honey marker and correlated REIMS features suggested direct detection of the honey marker. To date, there has been little work on understanding what adducts are formed during REIMS ionisation, though $[M\text{-}H_2O\text{-}H]^-$ have been suggested to be common in lipids (Song et al., 2020). The combination of direct analysis of the sample matrix with no clean-up and using a laser, and heat within the REIMS source might result in adduct formation that is different to that characterised for LC-MS.

3.7. Rapid analysis of honey

Verifying honey authenticity is a significant problem worldwide (Apimondia, 2019; Hegazi et al., 2022), and the balancing act of finding methods detailed enough or instruments sensitive sufficient to detect a wide range of adulteration methods yet fast enough to allow many samples to be analysed and cheap enough to not be inhibitory for the honey industry, remains constant. In this study, we applied REIMS, which is analogous to rapid metabolomics analysis and has previously been demonstrated as being adequate to distinguish between botanical origin of honeys and dilution with rice and corn syrup (Wang et al., 2019). Although an expensive initial investment, the running costs of REIMS are low, and with a laser interface as used in this study, has a low personnel burden. REIMS features can also be directly linked to specific honey components through the measurement of high-resolution mass, as demonstrated with leptosperin. However, more work is needed to identify other significant features in the REIMS spectra (Fig. 1). Other mass spectrometry-based systems have been proposed for honey analysis, including atmospheric solids analysis probe mass spectrometry (Loh, Lee, Stead, & Ng, 2022). Spectroscopic methods are also an area of active research, and near-infrared spectroscopy was able to discriminate between monofloral, multifloral and non-mānuka honey with 89% accuracy and could also distinguish between honey from different geographic locations within New Zealand (Truong, Reddy, Reis, & Archer, 2022). NMR is also a commonly applied method for honey authentication and can detect many of the known markers of interest for mānuka honey (Schievano et al., 2016; Yusoff et al., 2022). An advantage of direct metabolomics analyses such as REIMS and NMR is that they detect a wide range of features down to the mg/kg level, meaning that the addition of synthetic compounds to for example mimic the MPI mānuka honey panel, would not be sufficient to mask the wide range of lower concentration features. In common with both types of approaches is that they are dependent on authenticated samples to build up the

Table 4
Multivariate correlations of all REIMS features with markers for mānuka honey. Data were log-transformed prior to correlation analysis. Markers contributing to the multivariate models, along with their individual correlations are outlined in Supplemental Table 1.

DHA	
Multivariate REIMS negative mode	0.992
Multivariate REIMS positive mode	0.708
MGO	
Multivariate REIMS negative mode	0.961
Multivariate REIMS positive mode	0.708
HMF	
Multivariate REIMS negative mode	0.988
Multivariate REIMS positive mode	0.877
Leptosperin	
Multivariate REIMS negative mode	0.989
Multivariate REIMS positive mode	0.834
4-HPLA	
Multivariate REIMS negative mode	0.900
Multivariate REIMS positive mode	0.828
4-HPLA NZ only*	
Multivariate REIMS negative mode	0.993
Multivariate REIMS positive mode	0.753
2-MBA	
Multivariate REIMS negative mode	0.939
Multivariate REIMS positive mode	0.881
2′-MAP	
Multivariate REIMS negative mode	0.971
Multivariate REIMS positive mode	0.978
2′-MAP NZ only*	
Multivariate REIMS negative mode	0.989
Multivariate REIMS positive mode	0.717
3-PLA	
Multivariate REIMS negative mode	0.986
Multivariate REIMS positive mode	0.902

* Some of the markers were present at negligible concentrations in Australian honeys. Correlations were recalculated with New Zealand honeys only.

databases that are used to determine what is 'authentic', and that the larger the number of samples, and better defined they are, the greater the confidence for matching against the database. Due to the widespread fraud in the mānuka honey sector, using databases built solely on commercially available honey could be problematic.

3.8. Limitations

In this study, we have used a convenience sampling of commercially available honey labelled 'manuka', 'mānuka' or 'tea tree' from New Zealand and Australia. This may have led to some bias and while fraud within the market of origin is likely to be low, there are significant concerns about fraud, especially for mānuka honey. None of the honey samples fell outside of an expected range for any of the standard honey measurements, nor were any clear outliers based on PCA modelling of the REIMS data. Based on this we assumed that there were no adulterated honeys among those collected for this study. Larger sample numbers would help to improve the confidence in these findings, including a collection across several years. Including non-mānuka honey samples would also help to understand the variation between New Zealand and Australian mānuka honey samples in the broader context of honey from the nectar of other plant species.

A weakness in many studies aiming to detect specific markers for mānuka honey is how samples are collected and whether all *Leptospermum* spp honeys are analysed together as one group (e.g. honeys labelled 'manuka'), or *L. scoparium*-derived honeys are separated from other *Leptospermum* honeys. This distinction is, in most cases, a geographic one of comparing New Zealand (*L. scoparium*) to Australian (*Leptospermum* spp) honey, which can be controversial in relation to whether only *L. scoparium* derived honeys from New Zealand should be allowed to be labelled 'mānuka' or 'manuka', or whether the name 'manuka' can be applied to any *Leptospermum* honey containing MGO. Highlighting how chemical analysis can contribute to the differentiating *L. scoparium* compared to other *Leptospermum* honeys, several honey samples labelled as mānuka honey purchased in Singapore did not meet the MPI definition or contained below the lepteridine threshold set by the authors (Lin et al., 2020). In the present study, most Australian honeys did not meet the MPI definition for multifloral mānuka honey. Using REIMS as an independent measurement method, a considerable compositional difference between *Leptospermum* honey from New Zealand and Australia was detected, supporting findings based on the MPI markers. We cannot rule out that there is a difference in the processing and handling of the honey samples between countries contributing to this difference. HMF concentrations differed between countries with Australian honey having on average much higher HMF concentrations that New Zealand honey, suggesting the Australian samples have undergone excessive heating or have been stored for extended periods.

The use of REIMS to assess the honeys has the advantage of speed, as no preparation of the sample is required beyond mild heating to allow pipetting. However, the use of an energy source, in this study a CO_2 laser, to generate the aerosol or 'smoke', some of the metabolites in honey will be thermally altered or degraded. To this end, while some tentative identification is possible with REIMS, including likely direct detection of relevant markers including leptosperin, 3-PLA and 3-MBA, many features were possible to identify based on high resolution mass alone. REIMS is best utilised as a fingerprinting tool to screen for differences which can then be followed up using other analytical instruments such as GC–MS, LC-MS and NMR.

4. Conclusions

In a broad sample of commercially available *Leptospermum* honey samples, it was possible to use REIMS fingerprinting to differentiate between honey from New Zealand and Australia with similar MGO concentrations. The composition differed markedly, with over 50% of detected features varying between honey from the two countries. This supports previous literature that suggests that geographic origin is firmly imprinted in the metabolite composition of honey. While the exact identity of what is detected in honey by REIMS is not yet determined, the strong correlations between REIMS features and objective markers of mānuka honey suggest that what is detected is related to other methods used to measure metabolites in honey. Further work to determine how geographic variation within New Zealand impacts on REIMS fingerprints and comparisons between *L. scoparium* honey from Australia is required to refine potential markers of the country of origin.

CRediT authorship contribution statement

Alastair B. Ross: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Terry J. Braggins:** Formal analysis, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2023.100414.

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