INTRODUCTION
Recent scandals have highlighted that food fraud can also result in major food safety issues. Food fraud is a collective term which describes a substitution, addition, alteration or misrepresentation, deliberate and intentional, of food ingredients or of food packaging, or false or misleading statements formulated concerning a product for economic gain [1].

For example, in Europe foods labelled as beef were found to contain undeclared horse meat; in China, milk and infant formula were adulterated with nitrogen rich melamine, added to food products to increase their apparent protein content; both for monetary gain.

Adulteration of honey by syrups, sugars or flavour enhancers can make it cheaper to produce and extend shelf life. The purity of the honey can be deceptive. Isotope ratio mass spectrometry can be used to detect as little as 7% addition of corn syrup and cane sugar [2]. However, fraud due to mislabelling or false or misleading claims of botanical origin can be more difficult to detect.

In a high profile case in 2008 [3], the German food conglomerate ALW took Chinese milk powder and misrepresented it as milk powder produced in Germany to be sold in Russia. The product then moves to searching for markers of floral origin with Progenesis QI. The raw data acquired was loaded into Progenesis QI. In a parallel experiment, Rapid Evaporative Ionization Mass Spectrometry (REIMS) was used to analyze the honeys directly, without sample preparation or chromatography (Figure 8). The resulting MS spectra were evaluated in the same fashion as the UPLC-HDMS data within Progenesis QI.

RESULTS AND DISCUSSION

Figure 3 shows chromatograms of the authentic honey samples from different floral origins. The mass spectra were interpreted. Feature identification is much more easily facilitated with this type of data. Feature annotation or identification, prior to annotation features were subject to filtering databases – contained separate samples from different countries (Norway, Denmark, Lithuania, Poland and New Zealand) and years of collection contained separate samples from different countries. Each floral class was investigated using OPLS-DA with an integrated export into EzId software (Figure 9).

Some of the metabolites identified were highlighted as being able to differentiate the Manuka honey samples (Figure 7). These metabolites have been verified as Manuka markers previously [5]. The identification of other markers is ongoing.

Figure 4. PCA analysis of the authentic honey samples showing differentiation of the different groups (pool QC, buckwheat, heather, rape and Manuka).

Further investigation was made into the specific differences between the honeys by binary comparison. The differentiation between the two botanical origins is investigated using OPLS-DA by an integrated export into EzId software (Figure 9).

The raw data acquired was loaded into Progenesis QI. Data was examined to see if the REIMS data could provide discrimination between honeys of different botanical origins and to discover whether the same markers were present in the REIMS data as detected by UPLC-HDMS. Figure 10 shows that botanical discrimination between heather and orange honeys was possible using both strategies.

Data was examined to see if the REIMS data could provide discrimination between honeys of different botanical origins and to discover whether the same markers were present in the REIMS data as detected by UPLC-HDMS. The total number of features identified by UPLC-HDMS was much greater, which also afforded identification from fragmentation information. REIMS was much quicker with no sample preparation or chromatography required. The two techniques are complimentary. UPLC-HDMS could be used for in-depth characterisation coupled with REIMS for simple point of origin testing. Alternatively, REIMS could be used for rapid screening with selected samples submitted for further investigation by UPLC-HDMS. Twelve unique markers were common to the two workflows.

CONCLUSION

Feed fraud is a multi-billion euro concern across the globe

- A non-targeted, profiling approach, using UPLC-HDMS combined with multivariate statistics, has been shown to be capable of differentiating samples of honeys of different botanical origins
- Biologically significant information is obtained by comparing multiple samples using an all in one high-throughput guided workflow in Progenesis QI
- The Progenesis QI facile database search engine allows both confident and putative assignment of markers
- Making use of ion mobility gives “cleaner” mass spectra facilitating easier identification of markers
- Verification of markers is important using complimentary technology
- Direct analysis using REIMS could provide opportunities for testing at “point of entry”

References
5. Luth et al (2016). Classification of Manuka honeys by 2014/15 NMRI/2, J Adv. Food Chem. 64: 1751-1756. Some of the metabolites identified were highlighted as being able to differentiate the Manuka honey samples (Figure 7). These metabolites have been verified as Manuka markers previously [5]. The identification of other markers is ongoing.

Figure 7. Chromatograms showing botanical content in various types of honey using LC-MSEMS with MRHM.

Figure 8. Chromatograms showing botancial content in various types of honey using LC-MSEMS with MRHM.

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