APPLICATION NOTE

FT-IR Spectroscopy

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Detecting Manuka Honey Adulteration Using Mid-Infrared Spectroscopy

Introduction

Manuka honey is a premium product produced by bees collecting nectar solely from the Manuka plant, found growing throughout New Zealand.[1] Its popularity stems from the wide range of health benefits it provides, from soothing the symptoms of a common cold to aiding the healing of wounds and burns.[2] Manuka honey can be classified based on its Unique Manuka Factor (UMF) which indicates the level of antibacterial activity present in each batch.[3]

Each year, 1,700 tons of Manuka honey are produced in New Zealand and yet over 10,000 tons of honey labelled as Manuka are sold worldwide. With prices reaching £90 a jar and extensive health benefits being claimed, fraudulent and adulterated Manuka honey poses a serious threat to consumers.[4] It is, therefore, highly important that an accurate and reliable adulteration detection method is available for manufacturers to routinely test their Manuka honey.

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Common adulterants found in Manuka honey include beet, corn and rice syrups. The differing photosynthetic pathways in the plants used to produce these adulterants mean that different techniques are required to detect them.[5] For example, the official method to detect corn syrup in honey is by measuring the $^{13}C/^{12}C$ isotope ratio but this method does not detect beet or rice syrup.[6]

Mid-Infrared spectroscopy, on the other hand, with Adulterant Screen™ technology, can provide rapid detection of all three adulterants without the need for solvents or time-consuming sample preparation.

Existing targeted approaches for adulterant screening, using Mid-infrared spectroscopy, require a quantitative calibration to be developed for each potential adulterant. Alternatively, non-targeted screening approaches such as a SIMCA (Soft Independent Modelling of Class Analogy) algorithm can determine whether a sample has been adulterated but will neither identify nor quantify the adulterant. PerkinElmer's Adulterant Screen, on the other hand, provides a semi-targeted method which allows quick identification and estimation of adulteration levels.

Experimental

MIR spectra of pure Manuka honey and beet, corn and rice syrups were collected using a PerkinElmer Spectrum Two™ FT-IR spectrometer with the PerkinElmer Universal Attenuated Total Reflectance (UATR) accessory, using the parameters shown in Table 1. Prior to analysis, the samples were warmed in a hot water bath for one minute in order to lower the viscosity of the honey.

36 spectra of pure Manuka honey (six replicates from six different commercially available products with differing UMF values) and one spectrum of each adulterant (beet syrup, corn syrup and rice syrup) were collected for the Adulterant Screen method. The spectra were pre-processed using a first derivative baseline correction, as seen in Figure 2.

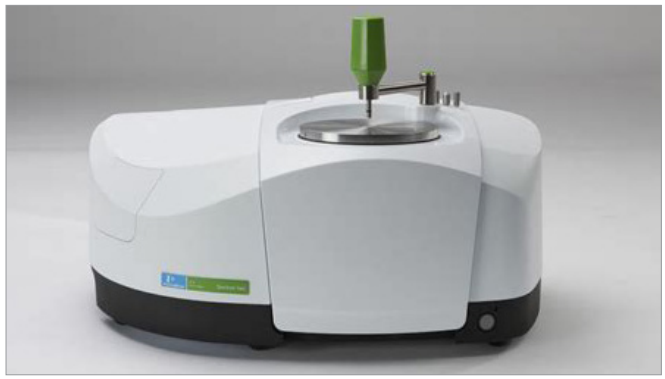

Figure 1. PerkinElmer Spectrum Two with Universal Attenuated Total Reflectance accessory.

Table 1. Scanning parameters for the analysis of Manuka honey and adulterant syrups.

Scanning Parameters	
Spectral Range	4,000 – 450 cm^{-1}
Resolution	8 cm^{-1}
Number of Scans	32

Additionally, 16 pure Manuka honey samples were spiked with each of the adulterant syrups over a range of concentrations from 5-90 % (w/w). Spectra of each adulterated sample, 100 % Manuka honey and 100 % adulterant were used to create quantitative Partial Least Squares (PLS1) models for each of the adulterant syrups using PerkinElmer Spectrum Quant™. 12 samples were used for calibration and six samples (25 %, 55 % and 85 %, including Manuka honeys with 5+, 10+ and 15+ UMFs) were used for independent validation of the model. Cross-validation was also carried out for each of the models, using the Leave-1-Out method. All spectra were pre-processed using MSC normalization and first derivative baseline correction with light noise reduction.

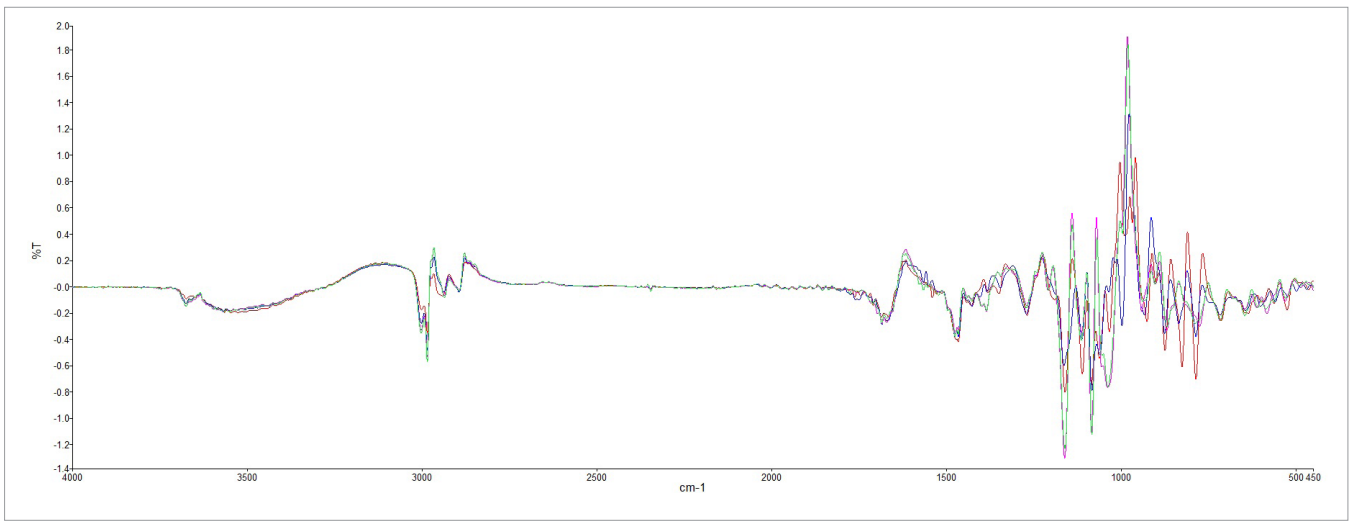

Figure 2. First derivative spectra of pure Manuka honey (red) and adulterant syrups (beet syrup (blue), corn syrup (pink) and rice syrup (green)).

Adulterant Screen

An Adulterant Screen method was created for identifying and semi-quantitatively estimating the level of adulterant in Manuka honey. Adulterant Screen is beneficial as there is no need for lengthy sample preparation and measurement of calibration standards. The user simply needs to create a library of unadulterated material spectra with as much natural variation as possible, such as samples from different batches or suppliers. Then, single scans of each adulterant must be collected in an adulterant library, which can be continually added to when required.

All 36 spectra of pure Manuka honey were inputted as 'material spectra' and the spectra of each of the three adulterant syrups were entered as 'adulterant spectra'. The method was tested using Manuka honey samples spiked with known levels of each adulterant. The results are shown in Table 2.

The Adulterant Screen method produced a "Fail" result in all cases. The algorithm correctly identified the adulterant syrup and gave an estimate of the level present in each sample. Samples of each of the pure Manuka honeys were also measured and these all produced a "Pass" result.

The detection limits are relatively low for each of the adulterant syrups. Although more expensive analytical methods can produce lower detection limits, economically motivated adulteration tends to be performed at a higher level in order to profit from it.

Adulterant Screen methods can also be incorporated into the Spectrum Touch™ software to provide a user-friendly interface for use by routine operators. Figure 3 shows the simple design of the workflow as it correctly identifies the adulterant syrup present. More detailed results are also provided, indicating the estimated levels of adulterant shown as a decimal.

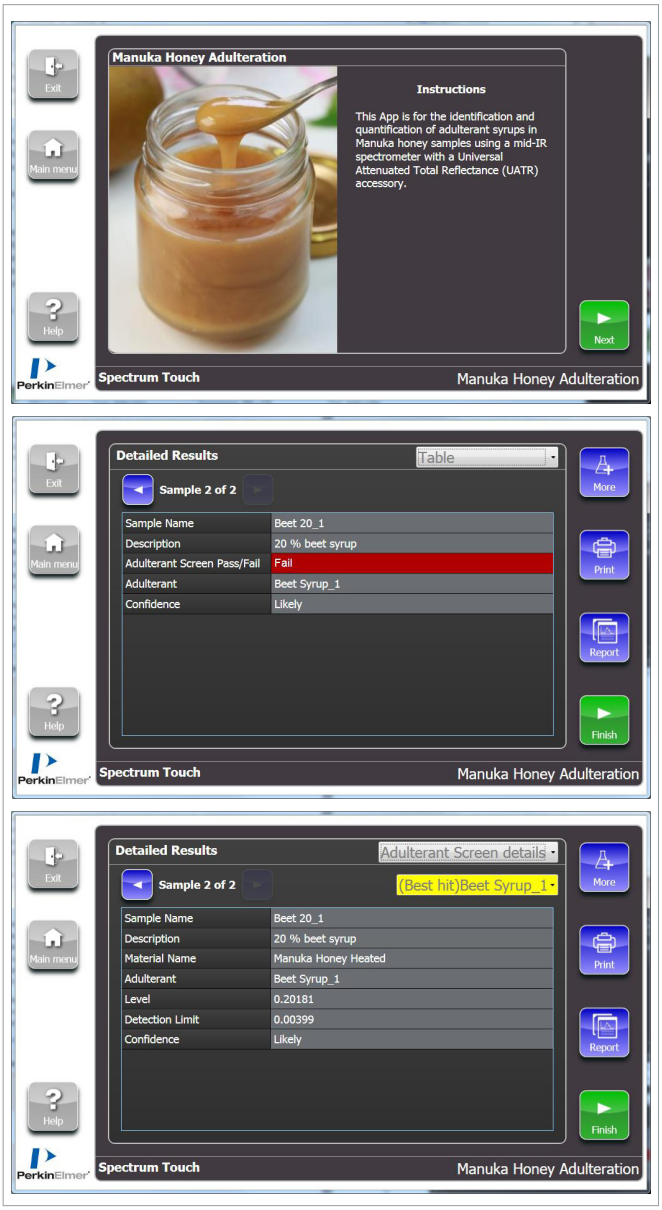

Figure 3. Example of Spectrum Touch Workflow and Adulterant Screen results for Manuka honey adulteration.

Table 2. Adulterant Screen results for a series of adulterated Manuka honey samples.

Sample Name	Predicted Level (%)	Detection Limit (%)	Adulterant Screen Pass/Fail
Beet Syrup (20 %)	20.18	0.40	Fail
Beet Syrup (10 %)	10.07		Fail
Beet Syrup (5 %)	3.82		Fail
Corn Syrup (20 %)	21.32	1.12	Fail
Corn Syrup (10 %)	13.67		Fail
Corn Syrup (5 %)	5.96		Fail
Rice Syrup (20 %)	20.18	1.52	Fail
Rice Syrup (10 %)	9.51		Fail
Rice Syrup (5 %)	2.70		Fail
Manuka Honey (100 %)	-	-	Pass

PLS1 Calibration Models

A PLS1 calibration model was also created for each of the adulterant syrups. Table 3 shows the regression data for all the calibration models. The R^2 values all exceed 99.9 % which indicates a very high level of correlation between the specified concentration of adulterant syrup and the concentration predicted by the model.

Table 4 highlights the average independent validation results for each of the models. The standard error of prediction (SEP) is relatively low for each of the models, indicating they have good prediction capabilities.

The results show that PLS1 calibration models are sensitive in predicting adulteration levels in Manuka honey. However, this method is very time-consuming as many calibration standards have to be prepared, covering all possible adulterant syrups as well as a range of UMF values and batches.

Conclusion

The results show that Mid-infrared spectroscopy with Adulterant Screen technology can provide a rapid screening technique for detection and identification of adulterant syrups in Manuka honey. The PLS1 calibration models provided accurate predictions for the level of adulteration present, but required time-consuming sample preparation and measurement. Adulterant Screen, on the other hand, rapidly identifies and provides a relatively accurate estimate of the level of adulterant present in the sample, without the need for calibration samples. If a new adulterant should arise, only one spectrum must be added to the 'adulterant library'. Adulterant Screen is, therefore, a more suitable method to use for routine checks for detection of adulterant syrups in Manuka honey.

References

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5. U. Gowik and P. Westhoff, *The Path from C3 to C4 Photosynthesis,* Plant Physiology, 2011, 155, 56-63.
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Table 3. Regression summary for adulterant syrup models (where SEC is standard error of calibration, SEP is standard error of prediction and CVSEP is cross validation standard error of prediction).

Adulterant Syrup	Number of PCs	R^2 (%)	SEC (%)	SEP (%)	CVSEP (%)
Beet	1	99.902	1.122	1.143	1.217
Corn	2	99.949	0.855	0.971	1.269
Rice	2	99.934	0.969	1.020	1.339

Table 4. Independent validation results from adulterant syrup models.

Adulterant Syrup	Average True Sample Property Value (%)	Average Predicted Sample Property Value (%)	SEP (%)
Beet	52.50	52.91	1.397
Corn	52.50	51.04	2.231
Rice	52.50	50.64	2.719

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