APPLICATION NOTE

FT-NIR Spectrometry

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Detection of Honey Adulteration Using FT-NIR Spectroscopy

Introduction

It is not uncommon to find high-value commodities such as foods to have compromised quality. These commodities can be adulterated by unscrupulous suppliers to increase their profit margins. Unfortunately, it is often difficult to determine if products have been adulterated.

A high-value product commonly associated with adulteration is honey. Adding corn syrup allows dishonest suppliers to maintain the sweet taste without a noticeable difference in the product. Without testing, it is hard to tell which honeys are adulterated and which are not. Traditional testing methods for adulterated honey can be lengthy and expensive. Fraudulent mislabeling of honey is also a major problem. FDA guidelines for labeling of honey state:

- If a food contains only honey, the food must be named "honey".
- If a food contains honey and any other ingredients such as sweeteners it must be labelled accordingly, for example, "blend of honey and sugar".
- The floral source can be stated, such as Clover Honey.
- Any product that is not pure honey cannot be labeled as "honey."

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Fourier Transform Near-Infrared Spectroscopy (FT-NIR) provides a quick, high quality testing method that allows for the detection of adulterants in honey. In order to optimize the effectiveness of the technique, various data modelling approaches were tested.

Data Analysis Approaches for Detection of Adulterants

The detection of adulterants in products can be either targeted or non-targeted. In targeted approaches, such as Partial Least Squares (PLS), the adulterant is a specific material that you are looking for within the product. This allows for a quantitative measurement of the amount of that adulterant, assuming a suitable calibration has been generated from a series of calibration standards. Each adulterant material will require a separate calibration. A typical non-targeted approach, such as Soft Independent Modelling of Class Analogies (SIMCA), will inform the analyst if the product does not conform to the expected material profile. It will indicate that the product may be adulterated, but it cannot say what it is adulterated with and by how much.

Spectrum 10's unique Adulterant Screen™ will inform the analyst when the product does not conform, identify the adulterant, and estimate the concentration of the adulterant without the lengthy requirement of running (multiple concentration) standards for each known adulterant and future adulterants. This allows for rapid deployment of initial Adulterant Screen methods and rapid method updating with new adulterants.

Experimental

NIR spectral data was collected on a PerkinElmer Frontier™ NIR spectrometer by pouring the honey sample into a Petri dish, placing the Petri dish onto the top of the NIRA II Reflectance Accessory, and placing a Transflectance Adaptor on top of the sample. Spectra were collected at 8 cm^{-1} resolution using a scan time of 30 seconds.

Spectra of the following pure samples were measured:

- Clover honey
- Wildflower honey
- Orange blossom honey
- Organic honey
- Corn syrup
- Rice syrup

Ten replicate spectra were measured for each of these pure materials.

In addition, dilutions of the pure material using corn syrup were prepared yielding the following concentrations:

- Clover Honey
  - ♦ 0%, 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 92%, 94%, 96%, 98%, 100%
- Wildflower Honey
  - ♦ 0%, 20%, 40%, 60%, 80%, 100%
- Organic Honey*
  - ♦ 0%, 20%, 40%, 60%, 80%, 100%

Additional sample dilutions were prepared as validation samples to test the methods: The wildflower, orange blossom, clover, and organic honeys were diluted two separate times, once with 10% corn syrup and once with 10% rice syrup.

Results

Figure 1 contains the scans of three different samples with varying concentrations of honey (0% honey is Corn Syrup). There are clear spectral differences between the samples at these high concentrations. The second derivative (Figure 2) simplifies the view to quickly identify differences and will also remove any baseline offsets or slopes from the data.

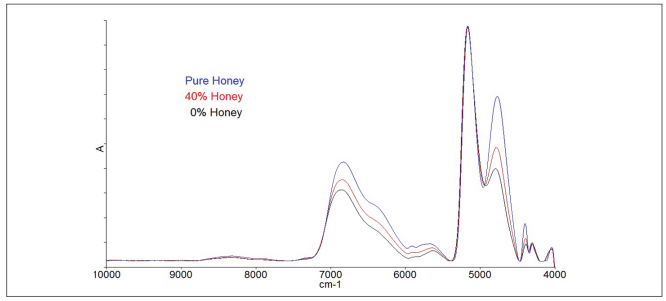

Figure 1. FT-NIR overlay demonstrating the typical spectra of honey.

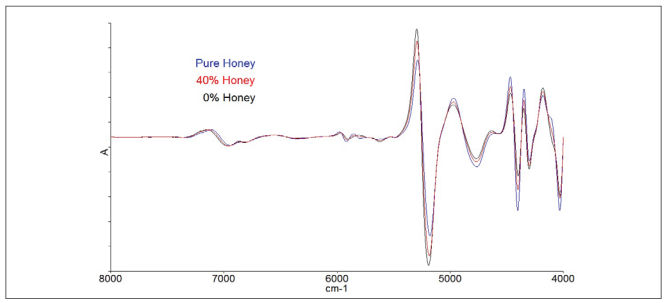

Figure 2. Second derivative spectra of samples in Figure 1.

A PLS quantitative model was generated from the clover honey/corn syrup standard mixtures. Figure 3 shows the calibration for the NIR estimated concentration versus the specified mixture concentrations for these standards. The data shows an excellent correlation indicating that PLS modeling may be successful in characterizing honeys with "known" adulterants.

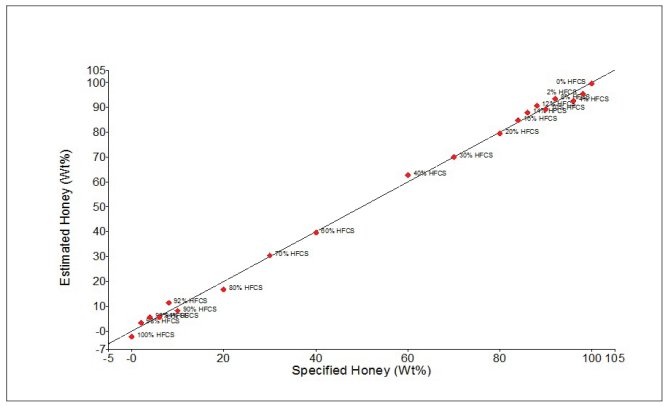

Figure 3. PLS model of honey dilutions with a line of best fit.

A further calibration was performed incorporating all of the standard mixtures from organic, clover, and wildflower honeys, shown in Figure 4. This calibration implies that the flower type has little impact for the samples and adulterants chosen in this model.

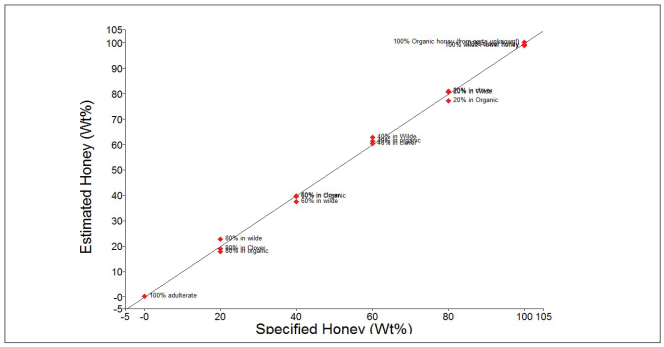

Figure 4. PLS model of three different honeys of the same dilution seen on the line of best fit.

Table 1. PLS result for honey validation sample.

	Validation Sample Concentration
Calculated Corn Syrup %	41.89 Wt%
Actual	41.25 Wt%
Difference	0.64

A validation sample of honey with known concentration of corn syrup, not included in the PLS model, was used to verify the honey calibration. Table 1 shows how the model quantified the unknown, with a difference of 0.64%.

A SIMCA model was generated by inputting 8 of the 10 replicate samples for each of the honeys using the spectral range 10,000-4,000 cm^{-1}, with 2nd derivative applied to the data. The remaining two replicates for each type of honey were used as an Independent Validation set along with a honey sample spiked with 10% of corn syrup.

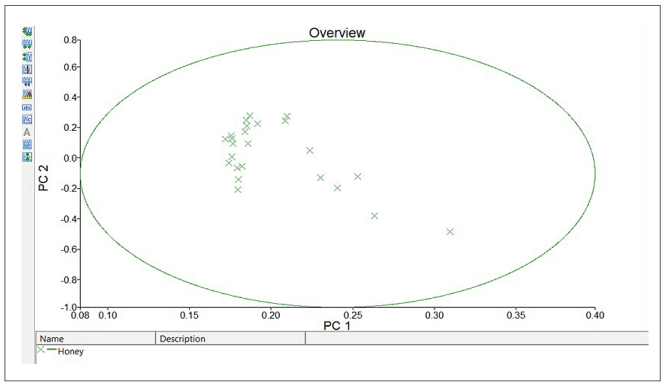

Figure 5. Principal Components plot for honey varieties.

Figure 5 shows the Principal Component (PC) plot of PC1 vs PC2 for the honey samples. All the clover, wildflower, and orange blossom honey spectra lay in within the boundary of the model. The results from the Independent Validation are shown in Figure 6.

	Sample I.D.	Specified Material	Identified Material	Result	Specified Material Total Distance Ratio
1	Clover honey 09	Honey	Honey	Passed	0.4805
2	Clover honey 10	Honey	Honey	Passed	0.5891
3	Wildflower Honey 09	Honey	Honey	Passed	0.6460
4	Wildflower Honey 10	Honey	Honey	Passed	0.6585
5	Orange Blossom Honey 09	Honey	Honey	Passed	0.5635
6	Orange Blossom Honey 10	Honey	Honey	Passed	0.7056
7	Clover Honey with 10% HFCS	Honey	Honey	Passed	0.7316

Figure 6. Independent validation results for honeys.

All of the replicate pure honey samples passed. However, the spiked sample also registered a pass result. The SIMCA method would require more work to try to determine an appropriate PASS/FAIL threshold.

An Adulterant Screen method was generated by inputting all of the pure honey spectra as "material spectra" and adding in high fructose corn syrup and rice syrup as "adulterant spectra". First Derivative pre-processing was applied within the method. The SIMCA method and the Adulterant Screen method were implemented in a Spectrum Touch™ application allowing for sequential analysis using SIMCA, followed by Adulterant Screen. The sample spiked with 10% high fructose corn syrup was tested using this Spectrum Touch method, as shown in Figure 7.

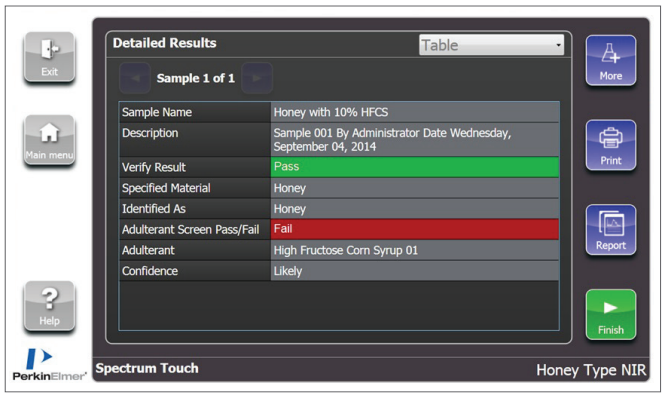

Figure 7. Results from Verify (SIMCA) and Adulterant Screen testing a 10% dilution of honey.

As detailed previously, the SIMCA analysis gives a false PASS result. However, Adulterant Screen correctly recognizes that the sample is adulterated with high fructose corn syrup.

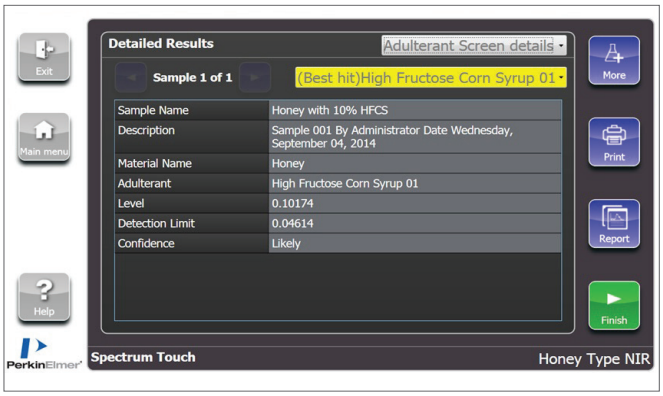

Figure 8. Detailed view of results from the Adulterant Screen of a 10% dilution of honey.

Figure 8 shows the detailed Adulterant Screen results for a honey sample diluted with corn syrup. The results show the estimated percentage of high fructose corn syrup the model found in the sample. The line labeled 'Detection Limit' indicates the minimum detection limit (about 4%) of this adulterant using this method. Adulterants with significantly different spectra from honey would be detectable at much lower limits.

Conclusion

The data included in this application note indicates that it is possible to use NIR spectroscopy to detect adulteration of honey. NIR sampling is quick and easy. If the adulterant is known, then quantitative analysis of the adulterant can be achieved with PLS modeling. However, this requires the lengthy preparation of calibration standards. Adulterant Screen can detect adulteration with better sensitivity than a SIMCA model and can recognize which adulterant is present and estimate the adulterant concentration without quantitative calibration standards. Finally, the method can be deployed in a simple user interface to allow use by routine operators.

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