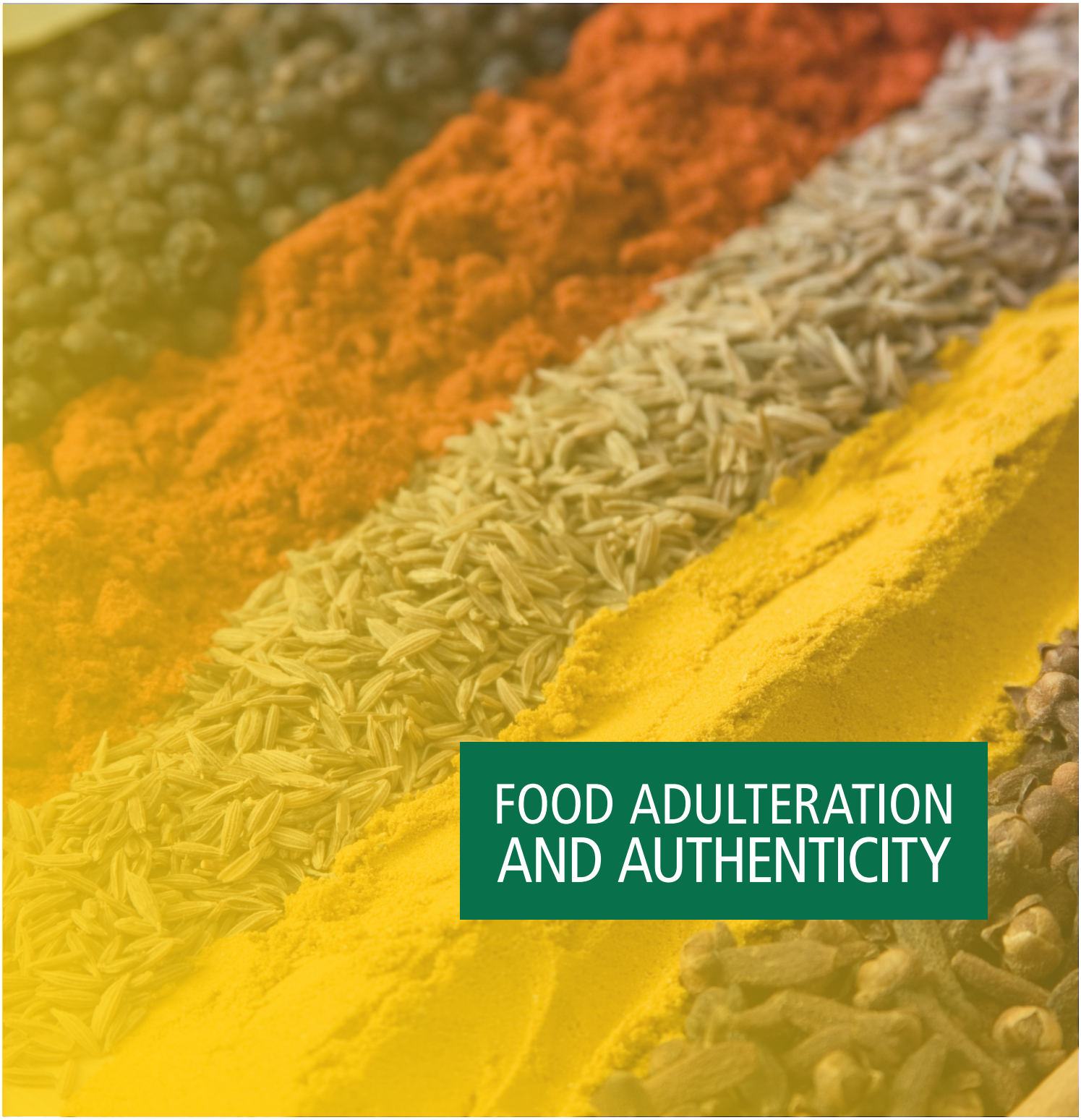


FOOD ADULTERATION AND AUTHENTICITY

Food Adulteration and Authenticity Compendium

PerkinElmer®
For the Better

Table of Contents

APPLICATION NOTE

Near- and Mid-Infrared Spectroscopy

Authors:

Kathryn Lawson-Wood

Ian Robertson

PerkinElmer, Inc.
Seer Green, UK

Comparison of Near- and Mid-Infrared Spectroscopy for Herb and Spice Authenticity Analysis

Introduction

Food authenticity and adulteration testing has taken on a new level of importance with consumers and producers, after recent food adulteration scandals. Food fraud negatively impacts consumer confidence and, more importantly, some adulteration can be harmful to health. Herbs and spices are one of the most commonly adulterated commodity products, with a prime example being oregano adulterated with olive and myrtle leaves[1].

Infrared (IR) spectroscopy is well recognized for the characterization of a wide range of materials and has advanced significantly over the last few decades[2]. This fast and cost-effective technique, when combined with a spectral library, can provide conclusive information about samples, and is ideally suited to both qualitative analysis of materials and quantification of components. Historically, IR instruments were generally quite large, required clean environments, and highly trained operators for interpretation of results. Advancements in sampling techniques, data analysis tools, ruggedness, and instrument portability have allowed easy analysis of a broad range of samples within a range of environmental settings[3].

PerkinElmer®
For the Better

IR spectroscopy combined with chemometric techniques such as Principal Components Analysis (PCA) is an extremely useful technique for food analysis. This application note compares the use of Mid- and Near-IR spectroscopy for authenticity analysis and adulteration detection in herbs and spices.

Infrared Spectroscopy

The IR region of the spectrum is generally split into three different sub regions:

- Far-IR: 400 – 30 cm^{-1}
- Mid-IR: 4000 – 400 cm^{-1}
- Near-IR: 14000 – 4000 cm^{-1}

The Far-IR region is primarily used for measuring inorganic molecules, thus not relevant for herb and spice analysis[4]. The Mid-IR region consists of fundamental absorption bands and provides a molecular fingerprint. This is especially beneficial for simple structural investigation and raw material ingredient or additive identification by library comparison. Absorption bands in the Near-IR region arise from overtones and combination bands of the fundamental bands in the Mid-IR region. The Near-IR region is particularly useful for food applications, including moisture, fat and protein content determination.

Comparing Mid- and Near-IR Spectroscopy for Herb and Spice Analysis

Foods are typically mixtures and encompass numerous sources of natural variation. In many cases in particular herbs and spices, IR spectroscopy combined with chemometric techniques such as PCA, which accommodate variation, can be used for sample classification.

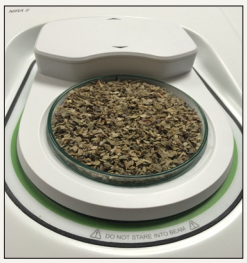
The most common sampling techniques for food samples are Attenuated Total Reflectance (ATR) for Mid-IR and diffuse reflectance for Near-IR. Diffuse reflectance may be used with Mid-IR but absorptions would be too strong without sample dilution. Table 1 compares Near- and Mid- IR methods for herb and spice analysis.

As a surface technique, ATR is highly suited to liquids and bulk homogeneous material measurement, but is less satisfactory for trace analysis, such as adulterant detection in herbs and spices. In diffuse reflectance mode, Near-IR radiation can penetrate much further into the sample, generating a long effective pathlength, yielding stronger spectra, thus making it more effective for adulterant detection analysis.

Mid-IR with ATR uses a small sample area and the resulting reproducibility of sampling is inconsistent due to pressure differences and different sample contact. Sample homogeneity of herbs and spices is typically poor, resulting in variations in spectra from different portions of the same sample. For leaf based materials, ATR generally analyzes a portion of a single leaf, making it unlikely to detect adulteration in the samples unless they are homogenized. Although Near-IR with diffuse reflectance requires much larger sample volumes, the large sampling area and ability to spin the sample during a scan results in better spatial averaging and more reproducible and representative sampling.

Figure 1 shows the Mid- and Near-IR spectra of ten different portions of a sage sample. Figure 2a-2c shows the standard deviation spectra for sampling different portions of the sample in un-powdered and powdered form. Given that a flat baseline in the standard deviation spectrum would represent no variance, large variance was observed between sampling in the Mid-IR spectra of the un-powdered form. Although less variance was observed when sampling powdered sage using Mid-IR (Figure 2b), it was still greater in comparison with the Near-IR standard deviation spectrum (Figure 2c). This demonstrates that homogenization is a required sample preparation step for measuring herbs and spices by ATR in Mid-IR spectroscopy. Comparing Figures 2a and 2c (the Mid- and Near-IR standard deviation spectra of the un-powdered sage), it is evident that there is significantly lower variance using Near-IR sampling.

Table 1. Near- and Mid-IR comparison for herb and spice analysis.

	Near-IR	Mid-IR
Sampling Type	Diffuse reflectance (non-destructive)	ATR (non-destructive)
Sampling Size	 Large (60-100 mm across)	 Small (2 mm across)
Sample Quantity	Several grams (generally not an issue as plenty of sample is usually available)	Milligrams
Sample Requirements	Inhomogeneous/homogeneous liquids and powders/solids	Homogeneous liquids and powders/solids
Sampling Reproducibility	High	Poor for inhomogeneous materials but high for liquids
Sampling Consumables	Glass petri dishes – transmit Near-IR radiation, are relatively low-cost, and no accessory cleaning is required	Cleaning with solvent and tissue
Sample Pathlength	~100 µm to several millimetres	A few microns (µm)

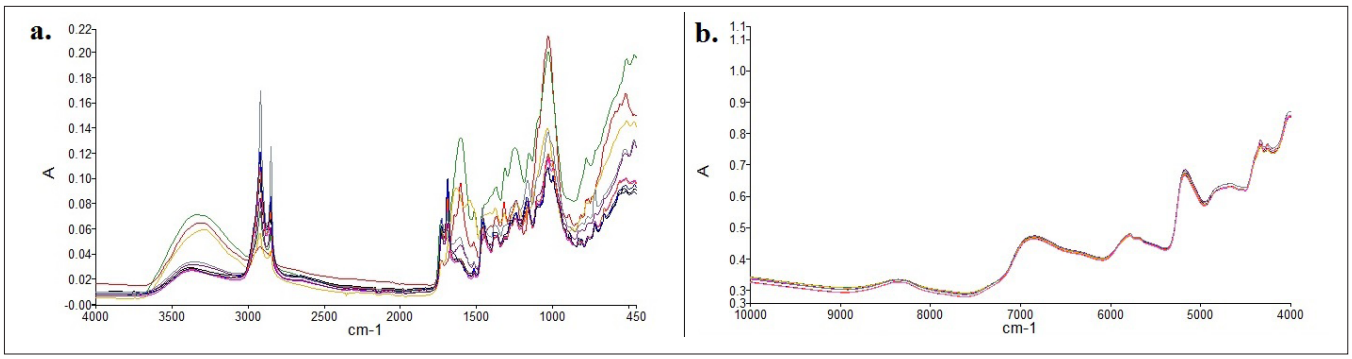


Figure 1. Ten different portions of the same sage sample: (a) Mid-IR spectra (b) Near-IR spectra.

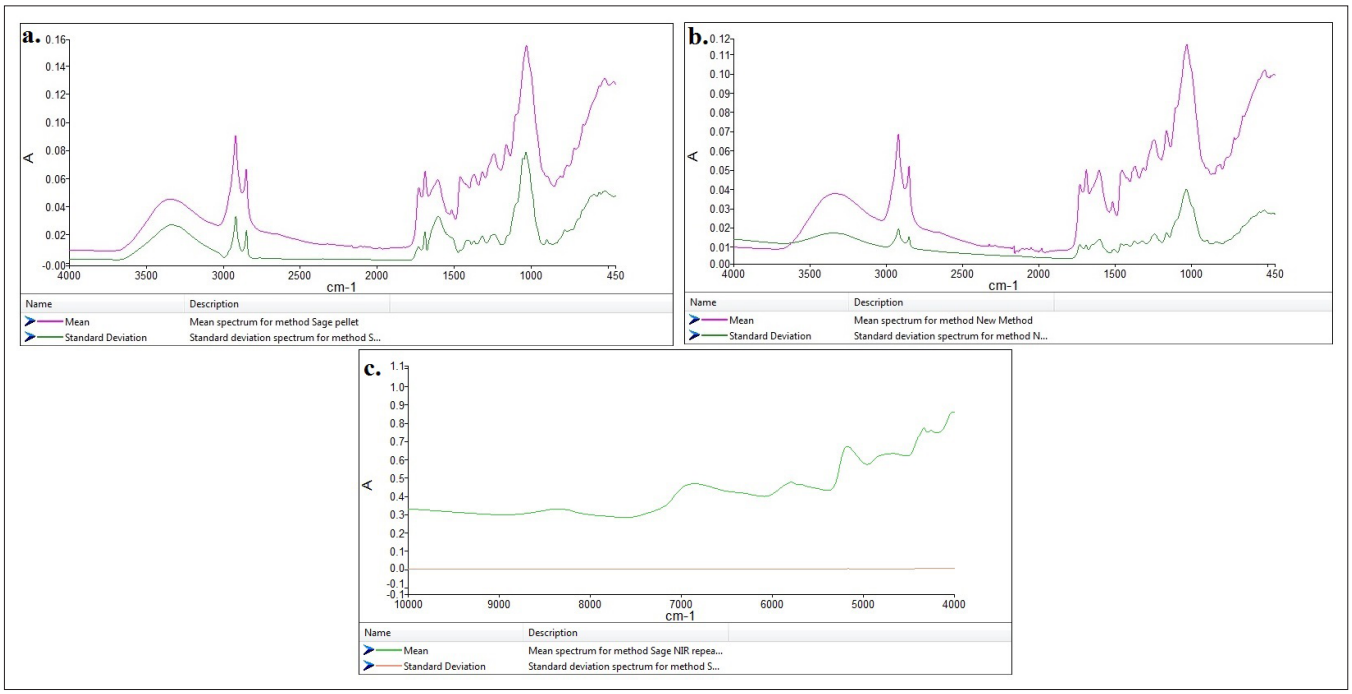


Figure 2. Mean and Standard Deviation of (a) Mid-IR spectra of unhomogenized sage; (b) Mid-IR spectra of powdered sage; (c) Near-IR spectra of unhomogenized sage.

Adulterant Screen Methods for Near-IR and Mid-IR

PerkinElmer's Adulterant Screen™ is an algorithm, using PCA, specifically designed for screening raw materials and products to determine authenticity, identify adulteration and estimate its levels[5]. It involves creating a spectral library of unadulterated samples for any given material. This library should include most of the possible sources of natural variation which arise from different suppliers, batches, sample pre-treatments and geographical origins. A library of pure adulterants is also required, but with just one spectrum for each adulterant. PerkinElmer Spectrum® Touch methods can readily be created for the Adulterant Screen methodology which encompasses a simple user interface; thus enabling routine operators to achieve rapid results for authenticity testing.

Twenty samples of sage and four samples of other materials (thyme, oregano, basil, and chives) were obtained from The Bart Ingredients Company (Bristol, UK). A spiked sample of 10% (w/w) thyme in sage was prepared to demonstrate the method. All samples were run on a PerkinElmer Frontier™ FT-IR instrument in both Near- and Mid-IR. Wavenumber ranges for Near- and Mid-IR spectra were 10,000 - 4000 cm^{-1} and 4000 – 450 cm^{-1} respectively. Scanning parameters were set to 8 cm^{-1} resolution and a one minute scan time. Separate Adulterant Screen Touch methods were created for each wavenumber range, using pure sage as the material spectra, and the other materials as adulterant spectra. After creating the Adulterant Screen methods, the 10% thyme in sage spiked sample was run four times, in Near- and Mid-IR, and the average thyme (%) levels estimated.

Adulterant Screen results for the 10% thyme in sage spiked sample are shown in Figure 3 for the Near-IR method. All samples failed Adulterant Screen, indicating adulteration. The average thyme level (%) was calculated to be 12.00%, illustrating reasonable levels of accuracy for this complex natural product, and the estimated detection limit was determined to be 10.29%. These detection limit orders of magnitude are expected with plant based materials, which are spectrally quite similar due to their cellulose content. However, adulteration with dissimilar materials would result in detection at lower concentrations. The 10% thyme in sage was not detected by the Mid-IR adulterant screen method. A potential explanation for this is the small ATR sampling area results in analyzing only a few flakes of the sample, making it problematic for inhomogeneous materials, and illustrating the difficulties in adulterant detection using Mid-IR spectroscopy.

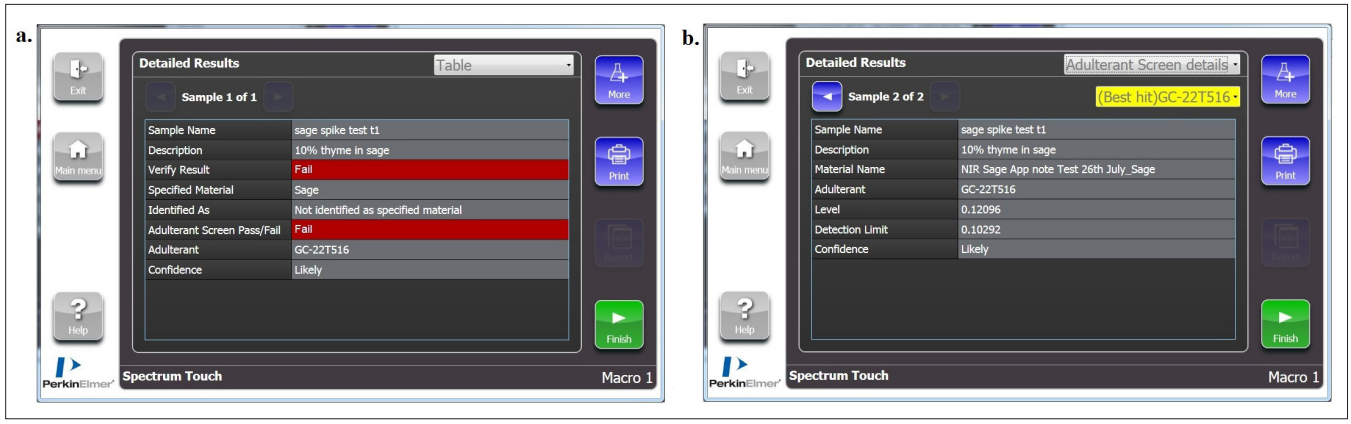


Figure 3. (a) Touch method results for 10 % thyme in sage sample for Near-IR (b) Detailed Adulterant Screen Touch results where GC-22T516 is thyme.

Conclusion

There is considerable scope for rapid on-site analysis of foods by using the combination of IR spectrometers with PCA to classify complex materials. This application note has shown that Near-IR spectroscopy is more suitable than Mid-IR for the analysis of herbs and spices and could also be applied to other inhomogeneous foods.

Near- and Mid-IR are both non-destructive techniques requiring no sample preparation. However, the longer effective pathlength and better spatial averaging available with Near-IR diffuse reflectance is beneficial for adulterant detection, and using glass sample containers allows for rapid analysis with no accessory cleaning required. Larger sampling areas and the ability to spin the material during Near-IR analysis results in more reproducible and representative sampling of food products with an inhomogeneous nature. Thus Near-IR spectroscopy is the ideal solution for herb and spice authenticity and adulteration detection analysis.

References

1. Black, C., et al. *A comprehensive strategy to detect the fraudulent adulteration of herbs: The oregano approach.* 2016, s.l. : Food Chemistry, 2016, Vol. 210.
2. Sibilia, J. *A Guide to Materials Characterization and Chemical Analysis.* s.l. : Wiley-VCH, 1996.
3. Brown, S. Analysis of the State of the Art: Infrared Spectroscopy. *Spectroscopy.* [Online] 2015. http://www.spectroscopyonline.com/analysis-state-art-infrared-spectroscopy.
4. Stuart, B. *Infrared Spectroscopy: Fundamentals and Applications.* s.l. : John Wiley & Sons Ltd, 2004.
5. PerkinElmer Health Sciences. *DairyGuard: Augmenting Nutritional Testing of Milk Powder with Adulterant Screening.* Shelton : s.n., 2013.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

013102A_01 PKI

APPLICATION NOTE

FT-IR Spectroscopy

Authors:

Ian Robertson

PerkinElmer, Inc.
Shelton, CT

Advantages of Adulterant Screen for Detection of Olive Oil Adulteration by Attenuated Total Reflectance (ATR) FT-IR

Introduction

Olive oil is an increasingly popular food product worldwide, with consumption in the U.S. alone having increased by about 50% in the last 10 years. Over three million tons annually of olive oil are produced worldwide, with approximately 75% of this being produced in Spain, Italy, and Greece. The U.S. now imports over 300,000 tons of olive oil annually.

Olive oil is considered to be healthy edible oil and is linked to the low incidence of heart disease associated with a Mediterranean diet. It is low in Saturated Fatty Acid (SFA) and Polyunsaturated fats (PUFA) but high in the healthier Monounsaturated fats (MUFA), known to lower cholesterol.

Extra Virgin Olive Oil (EVOO) is a premium product that can command a higher price than "standard" olive oils. This makes it highly susceptible to fraudulent activity. A report by the E.U. Committee on the Environment, Public Health, and Food Safety says olive oil is among the products most prone to food fraud. There were 267 oil adulteration incidents reported to the U.S. Pharmaceutical Food Fraud Database, with the vast majority occurring over the past three years.

Adulteration of EVOO with lower quality olive oils, or other lower cost edible oils, is frequently reported in the media. The most common adulterants include: hazelnut oil, sunflower oil, soybean oil, corn oil, rapeseed oil, and olive pomace oil. Fraudulent activities, such as dilution or even substitution with other lower cost oils containing additional chemicals, that enable the oil to appear to be of higher quality oil and pass routine screening tests are on the rise.

PerkinElmer®
For the Better

This application note describes a fast, simple, low-cost solution to screen olive oils for adulteration.

Materials and Methods

Mid-infrared spectroscopy is a well-established technique for the analysis of edible oil samples. The PerkinElmer Spectrum Two™ FT-IR, a high-performance compact FT-IR instrument utilizing the modern ATR sampling technique, offers fast and easy measurements of samples within the food industry. Diamond™ ATR accessories, such as the PerkinElmer Universal ATR (UATR), are extremely robust and allow the instrument to be used in the harshest of laboratories or even in remote environments. The Diamond ATR crystal requires only a very small volume of the sample to be tested and can easily be cleaned between samples, in situ, using laboratory tissue and a small amount of a suitable solvent, such as hexane for edible oils.

In this study the PerkinElmer Spectrum Two, equipped with a UATR sampling accessory, has been used to analyze a series of pure and adulterated olive oils and common adulterant spectra. A typical olive oil spectrum is shown in Figure 2. Spectra were recorded at 4 cm^{-1} resolution with a scan time of one minute per sample.

The prominent features in the spectrum are the bands in the region of 2930 cm^{-1} due to the –CH- stretch of the hydrocarbon chains and in the region of 1740 cm^{-1} due to the carbonyl groups in the triglyceride.

Discriminating Olive Oil from Other Edible Oil Types

The infrared spectra of different edible oils will be similar, only varying by the constituent chains on the triglyceride backbone, since their molecules contain the same chemical groups. However, there are small, observable differences between the different oil types. Figure 3 shows the ATR spectra of three different oil types: olive oil, sunflower oil, and rapeseed (canola) oil.

These spectral differences are significant enough to be able to develop a classification method for these different oils. There are a variety of ways to classify materials based on their infrared spectra. For this type of problem Soft Independent Modeling of Class Analogy (SIMCA), a Principal Components Analysis (PCA) based method, is a good approach to take. Building a SIMCA method requires the measurement of a variety of samples for each type of material you wish to classify. The calibration set of samples should cover all sources of variation normally encountered for that particular material, such as different sources, different batches, or different manufacturing processes. The method will build individual models to completely characterize each of the materials. Each material, in this case the individual oil types, generates its own cluster in this model that should be separated from the other clusters calculated for the other materials being classified. A SIMCA model has been generated for the three types of edible oils in this study. Figure 4 shows the SIMCA model with each oil having its individual cluster, clearly separated from those of the other materials.

Figure 1. The PerkinElmer Spectrum Two and UATR.

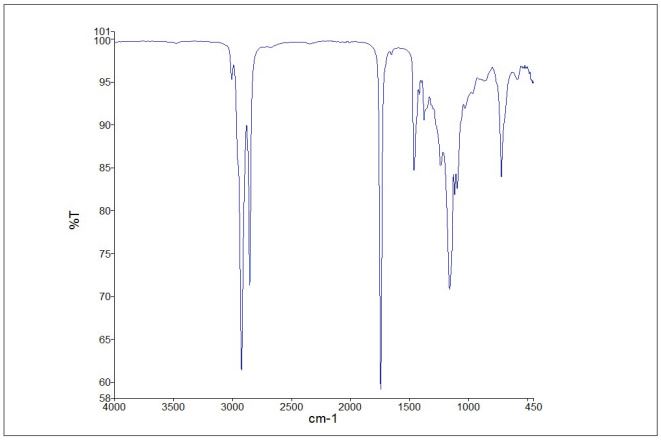


Figure 2. Diamond ATR spectrum of olive oil.

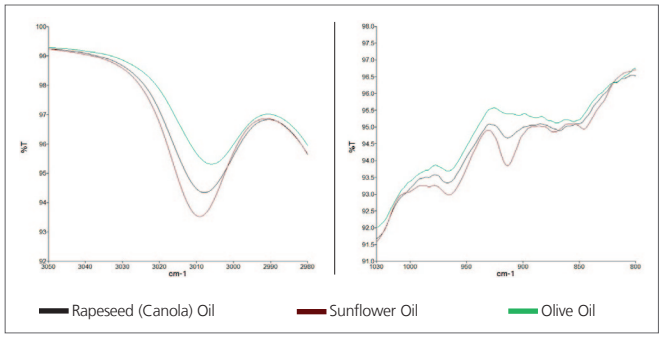


Figure 3. Spectral differences between olive oil, rapeseed oil and sunflower oil.

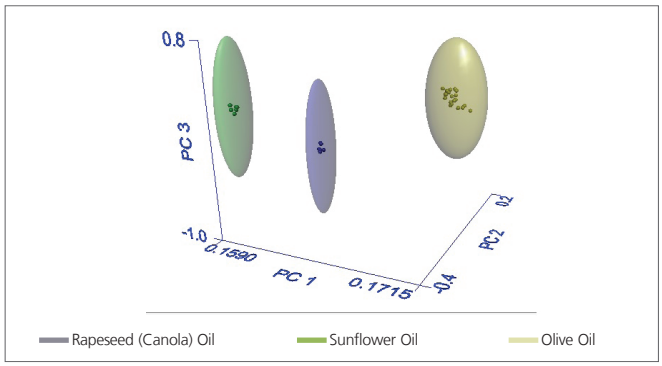


Figure 4. SIMCA model for three edible oil types. Olive oil, rapeseed oil, and sunflower oil.

Classifying a material consists of measuring the IR spectrum and using the SIMCA model to predict to which cluster the spectrum belongs. If the spectrum does not fall into one of the three classes of materials then it is likely to be a different material or contaminated/adulterated oil. Further data investigation would be required to determine the reason that the sample has failed the test.

Quantifying Levels of Known Adulterants in Olive Oil

If the identity of the adulterant is known then it is possible to quantify the amount of adulterant present. This involves the preparation and measurement of the IR spectra of standard mixtures of the olive oil with the adulterant oil. The IR spectra for a series of standards are shown in Figure 5.

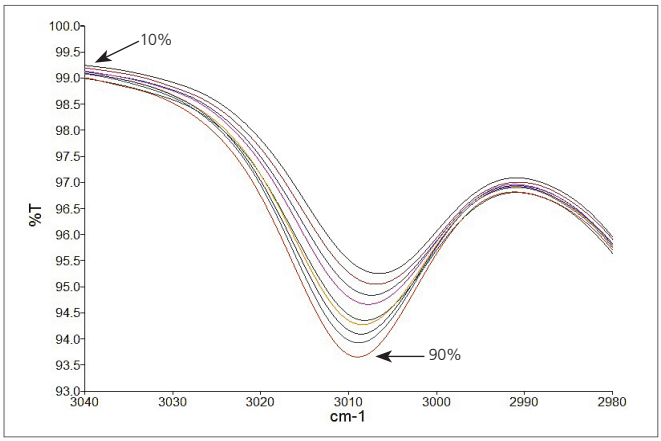


Figure 5. Standards from 10% - 90% Sunflower Oil.

Partial Least Squares (PLS1) Calibrations have been generated for mixtures of olive/sunflower oils and olive/rapeseed oils ranging from 0 to 100% olive oil. The calibrations are shown in Figure 6.

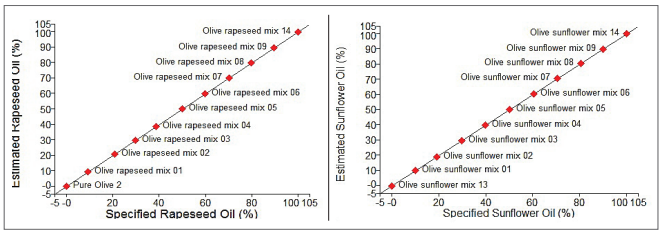


Figure 6. PLS1 Calibrations for Olive/Rapeseed and Olive/Sunflower oils.

An independent validation set of three samples were used to test the calibration model. The validation plot is shown in Figure 7.

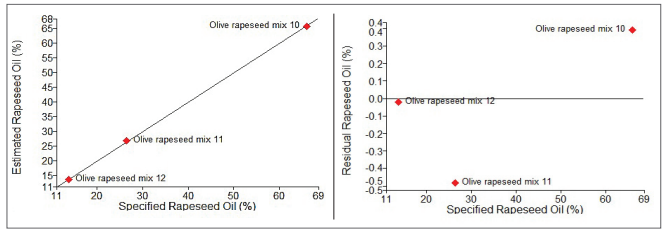


Figure 7. Independent validation samples for olive/rapeseed mixtures.

Adulterant Screen™ Algorithm for Detecting "Known" and "New" Adulterants in Olive Oil

The two statistical approaches taken so far would allow for:
a.) checking that the material is the correct material (SIMCA) and
b.) quantifying the amount of a single, known adulterant (PLS).

An alternative approach is available using an Adulterant Screen Algorithm. The approach is simple:

1. Generate a library of unadulterated material samples spectra exactly as for SIMCA. This library should span as much as possible the natural variation of the material, due to differences between batches, suppliers or processing parameters, etc.
2. Generate spectra of adulterants of concern. These spectra should be of the pure adulterant material, not mixtures. (As new adulterant materials emerge these can easily be added to the adulterant library in the future.)

These two sets of spectra are registered in the software, and the method is ready to use.

In this study, a series of 24 olive oil spectra were measured from commercially purchased oils. These 24 spectra were used to generate a library of the unadulterated material. The objective of this study was to specifically look for adulteration with either sunflower or rapeseed oils. Single spectra of the two adulterants were measured and stored with the method. The Adulterant Screen method was tested using samples adulterated with known concentrations of the other oil types and also with pure olive oil. The results are shown in Table 1.

Table 1. Adulterant Screen results for a series of method validation standards.

Sample Name	Adulterant	Level	Unidentified Components	Adulterant Screen Pass/Fail
Sunflower 18.66% Std	Sunflower Oil	0.19208	Probable	Fail
Sunflower 68.80% Std	Sunflower Oil	0.69011	Probable	Fail
Sunflower 38.10% Std	Sunflower Oil	0.38183	Probable	Fail
Sunflower 100.0% Std	Sunflower Oil	1.00328	Probable	Fail
Rapeseed 66.02% Std	Rapeseed Oil	0.64944	Probable	Fail
Rapeseed 26.41% Std	Rapeseed Oil	0.26367	Probable	Fail
Rapeseed 13.79% Std	Rapeseed Oil	0.14083	Probable	Fail
Rapeseed 100.0% Std	Rapeseed Oil	0.99191	Probable	Fail
Pure Olive Oil	No Adulterants	-	Unlikely	Pass

In all cases, except the pure olive oil, the adulterated samples generated a "Fail" result indicating the presence of an adulterant. Not only does the Adulterant Screen algorithm correctly identify the adulterant, but it also gives an estimated level of that contaminant without the requirement for running quantitative calibration standards. The level of the contaminant is reported as the proportion of the total spectrum contribution arising from that component. The results table demonstrates the ability of this algorithm to classify like SIMCA and additionally provide approximate estimates of concentration of the adulterants without the need to generate extensive quantitative models.

When a sample spectrum is scanned, the algorithm first compares it to a PCA model generated from the reference materials. This model is then augmented with each of the adulterant spectra in turn. If including a given adulterant in the model greatly increases the fit of the sample spectrum, it is likely that the adulterant is actually present in the sample.

Figure 8 shows the residuals observed from the analysis of 13.79% rapeseed validation standard.

Note: the spectral region from 2450-1850 cm^{-1} (the region where the diamond absorptions due to the Diamond ATR are intense) was excluded from the method.

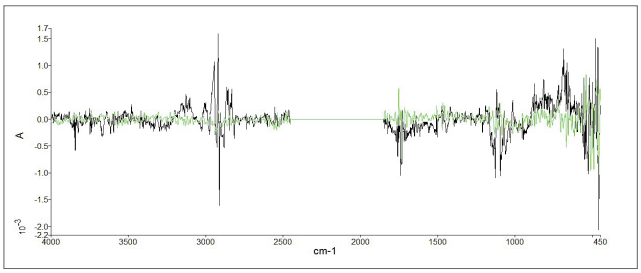


Figure 8. Spectral residuals before (black) and after (green) fitting adulterants.

In this case the residuals are significantly decreased by fitting the spectrum of the pure rapeseed oil indicating the presence of that adulterant in the sample.

Summary

ATR-FT-IR on the Spectrum Two allows for a fast, easy, and low-cost method for screening olive oil samples for adulterants. The information required from the analysis will determine which will be the most appropriate data analysis method to use. Data has been demonstrated using three different approaches – SIMCA , PLS, and Adulterant Screen. These are summarized below:

SIMCA – Is the product what it says it is and does it fall within the expected variation within that class of material? If not, further data analysis will be required.

PLS - For known adulterants it is possible to generate complete quantitative calibrations by preparing suitable standard mixtures. This will give accurate quantitative results.

Adulterant Screen algorithm – Is the product what it says it is and has it been adulterated? If adulteration is likely then try to identify the adulterant from known adulterants and give a semi-quantitative measure of how much of the adulterant is present.

The Adulterant Screen algorithm offers significant benefits over the other two approaches:

Faster method development

- The Adulterant Screen algorithm simply requires the collection of the spectra of the unadulterated material and the known adulterants.

Simple upgrade of methods

- When new potential adulterants are identified they can simply be added to the library of adulterant spectra.

Greater sensitivity than SIMCA

- Achieved by utilizing a library of spectra of potential adulterants.

Whichever statistical approach is utilized it can be deployed using a Spectrum Touch™ method, employing a simple user interface for the routine operator. Figure 9 is an example of the results screen for an adulterated sample.

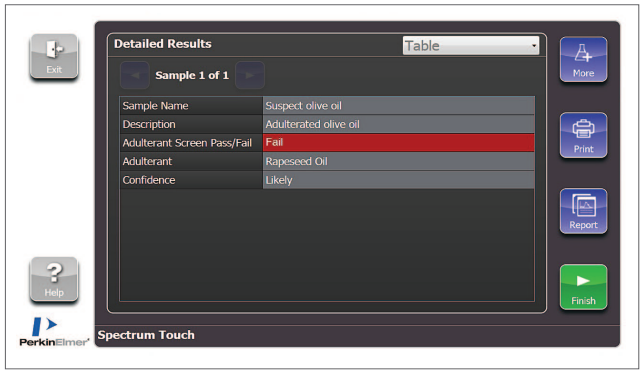


Figure 9. Spectrum Touch software showing result from Adulterant Screen.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

011735A_01

APPLICATION BRIEF

FT-NIR Spectrometry

Author:

Ian Robertson

PerkinElmer, Inc.
Shelton, CT

Rapid Testing for Adulteration of Yogurt Candy using Near-infrared Spectroscopy and Adulterant Screen

Introduction

Melamine is an adulterant commonly found in milk, as it can increase the nitrogen content. Therefore, its apparent protein content, resulting in a better market price. Melamine adulteration can be fatal, as was highlighted in 2008 when six infants died due to melamine adulteration in milk powder and thousands were sickened in China. Consequently, there have been stricter regulations globally and improved testing methods including the use of the PerkinElmer DairyGuard™ instrument for powdered milk testing.

However, cases of melamine adulteration are still appearing in other products. This year in Guangdong Province, China, 25 tons of yogurt candy tablets were seized as they were found to contain melamine. What follows is a description of a near-infrared (NIR) testing method of yogurt candy for melamine adulteration.

PerkinElmer®
For the Better

Experiment

Four different flavors of commercially-available yogurt candy were purchased (peach, cherry, blueberry, and tropical). The samples of yogurt candy for testing were ground into a powder and placed in a Petri dish. Spectra were collected on a PerkinElmer Frontier™ NIR spectrometer in reflectance using the sample cup spinner on the NIRA II sampling accessory at a spectral resolution of 16 cm^{-1} using 32 scans. Several replicate samples of the yogurt candies and the spectrum of pure melamine were added into Adulterant Screen™ as "Material" and "Adulterant" spectra, respectively. A sample of a mixture of the different yogurt candies was prepared using equal amounts of each flavor. The NIR spectra of melamine and the mixture of yogurt candies are shown in Figure 1.

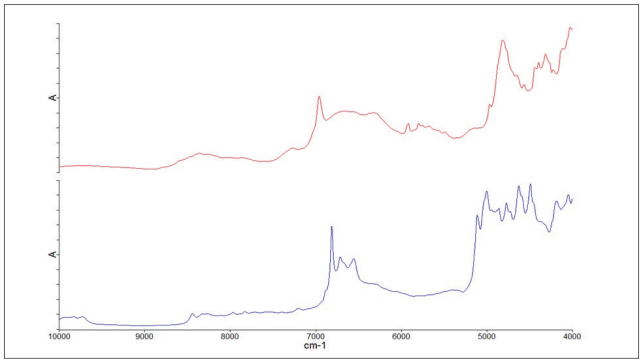


Figure 1. NIR spectra of melamine (blue) and mixture of yogurt candies (red).

The mixture of yogurt candies was spiked with melamine at 9.8%, 1% and 0.2% w/w levels and the spectra measured.

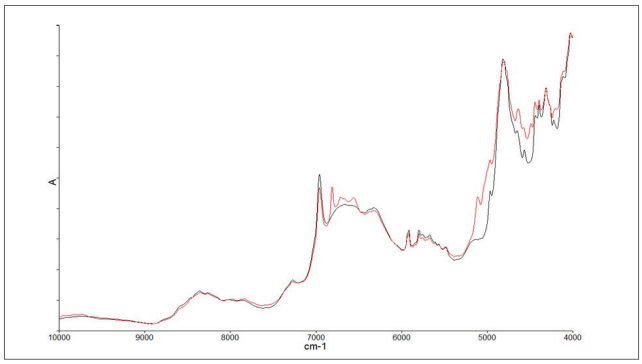


Figure 2. Spectra of yogurt sample with 9.8% melamine (red) and yogurt (black).

Figure 2 shows spectral features related to melamine present in the yogurt sample containing 9.8% melamine. The Adulterant Screen method was used to predict the presence and level of melamine in the samples, shown in Table 1. The close correlation between the measured amount and the predicted amount of melamine in the yogurt candy indicates that this Adulterant Screen method can be used to accurately predict the level of adulterant contamination.

Table 1. Adulterant Screen results for spiked samples.

Sample Name	Adulterant	Estimated Level (%)	Confidence
0.2% Melamine	Melamine	0.275	Likely
1% Melamine	Melamine	1.358	Likely
9.8% Melamine	Melamine	10.004	Likely

The Adulterant Screen method generates residual spectra from the analysis; showing the residual spectrum before adding in the adulterant spectrum, and the residual spectrum after adding in the adulterant spectrum. The spectral bands in the residual spectrum should decrease with the addition of the adulterant. Any remaining residual features are not accounted for by the method. Adulterant Screen allows for multiple adulterants to be detected and their concentrations estimated. The residual spectra from the analysis of the 0.2% melamine sample are shown in Figure 3.

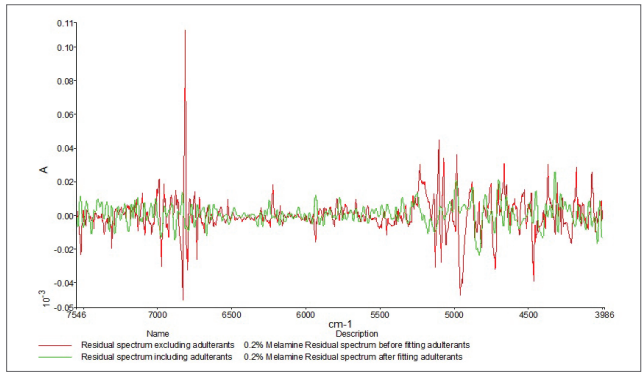


Figure 3. Residual spectra from Adulterant Screen for the 0.2% Melamine sample. Excluding adulterants (red), Including adulterants (green).

The addition of melamine into the Adulterant Screen model significantly reduces the residual spectrum, indicating the presence of melamine as an adulterant.

Conclusion

NIR spectroscopy with Adulterant Screen is a quick and simple method for detecting melamine adulteration in yogurt candy. The software is able to accurately predict the concentration level of melamine and identify any new adulterants. Rapid deployment of the method can be achieved for yogurt candy and similar products.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

011962_01 PKI

APPLICATION BRIEF

Near-Infrared Spectroscopy

Author:

Ian Robertson

PerkinElmer, Inc.
Shelton, CT

Use of NIR Spectroscopy and Adulterant Screen for the Detection of Common Adulterants in Milk

Introduction

The value of milk on the open market is linked to its protein content, and standard methods for protein analysis rely on a simple nitrogen assay, with the protein concentration inferred from the nitrogen content. Consequently, the addition of chemicals rich in nitrogen, such as urea, can artificially increase the apparent protein content and thus the price demanded. Urea occurs naturally in milk and is typically present at levels of about 0.02% - 0.05%. Higher levels of urea in milk are present only in cases of adulteration. Cane sugar is another known milk adulterant used to increase its carbohydrate content and weight. This allows extra water to be added into the milk without detection from a standard lactometer test for milk quality.

NIR spectroscopy coupled with PerkinElmer's Adulterant Screen™ is shown here to be capable of detecting adulterants intentionally or accidentally added to milk.

PerkinElmer®
For the Better

Method

Spectra of a variety of milk samples were collected on a PerkinElmer Frontier™ NIR spectrometer in transflectance using the NIRA II sampling accessory. The set of samples was selected in order to cover adulteration within a broad range of different types of milk, and included full fat, semi-skimmed, skimmed, lactose-free, and organic varieties. These spectra were defined in Adulterant Screen as our set of Material Spectra and represented "good samples." A spectrum of cane sugar, urea, and a spectrum of a 10% aqueous urea solution were measured as adulterants.

A full-fat milk sample was spiked with urea to give a urea concentration of 1% w/w. The spectra of the milk sample and the urea-spiked sample are shown in Figure 1.

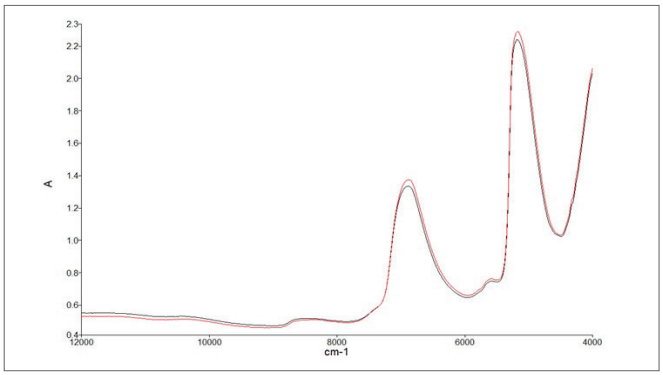


Figure 1. Spectra of whole milk (red) and milk spiked with 1% urea (black).

Although the spectra appear to be very similar, the application of a second derivative function shows that there are clear differences in the spectral region associated with urea absorptions as shown in Figure 2, thus allowing for the detection of urea in an adulterated milk sample.

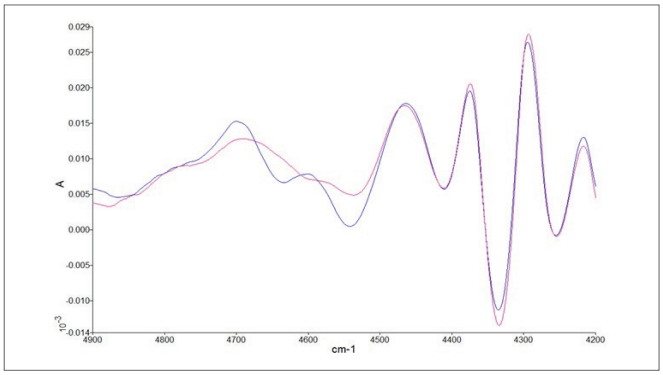


Figure 2. Second derivatives showing differences between milk (purple) and sample spiked with urea (blue).

The normal process for finding adulterants simply requires the measurement of a sample of the adulterant to provide a reference for subsequent comparison with the sample spectra. However, in the case of urea, the infrared spectrum changes significantly in the presence of water, resulting in the urea adulterant being incorrectly determined. The spectra of urea powder and urea solution (with the water subtracted) are shown in Figure 3.

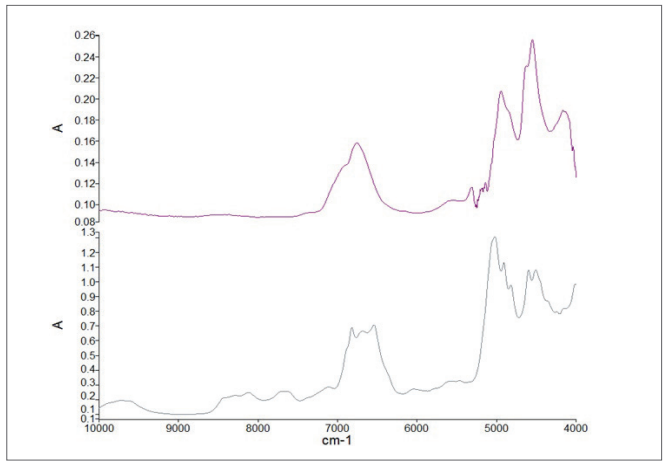


Figure 3. Spectra showing differences between 10% urea solution with water subtracted (top) and urea powder (bottom).

The urea solution spectrum with water subtracted is a more representative spectrum of urea in aqueous samples, such as milk, and should be used as the adulterant spectrum. This spectrum was then normalized to represent a 100% urea standard and added to the list of adulterants for this method. A spiked full-fat milk sample could then be checked for adulterants using Adulterant Screen. The result for the spiked sample is shown as Figure 4.

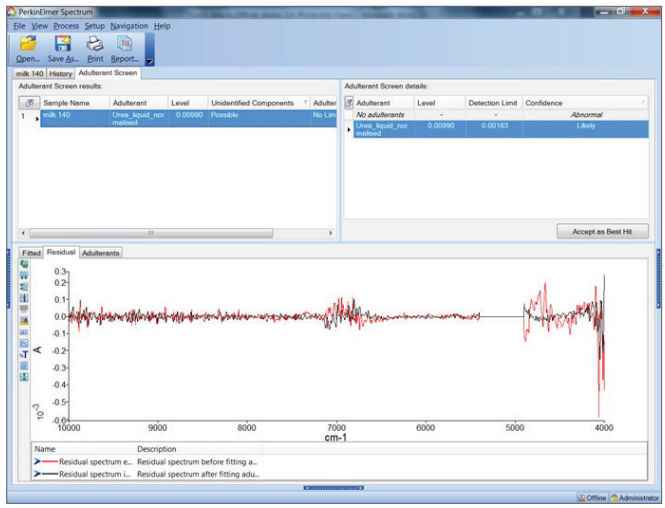


Figure 4. Adulterant Screen result for full-fat milk sample spiked with 1% urea.

The Adulterant Screen result that appears here shows the spectral residual when the unknown is tested against the model for the "good spectra" and the improvement achieved in reducing the residual when the spectrum of the adulterant (urea) is added. Adulterant Screen will also generate an estimated concentration of the adulterant and a detection limit. The estimated concentration of urea in this sample is 0.990%, very close to the known 1% concentration.

A different full-fat milk sample was spiked, but this time with sugar to give a 10% and 20% w/w of sugar. Adulterant screen was applied, and the results are shown in Table 1.

Since the cane sugar spectrum was measured in reflectance on the powder and the milk measurement is performed in transflectance on the liquid there are differences between the expected and observed levels of the adulterant. Therefore, the adulterant spectrum for sugar was normalized based on a known 10% sample. The limit of detection for cane sugar as estimated by the software is 3.5%.

Conclusion

Adulterant screen has been shown to be an effective method in detecting the adulteration of milk. Normalization of adulterant spectra may be required for some samples due to spectral changes that occur in solution. Nevertheless, NIR with Adulterant Screen is a fast and simple technique to use for the detection of adulterants. Additional adulterants can be readily added to the method by simply measuring the spectrum of the pure adulterant; thus providing a dynamic platform for adulterant screening.

Table 1. Adulterant screen results for milk spiked with sugar.

Sample Name	Adulterant	Level	Confidence	Material Fit
10% sugar	Cane sugar	0.10529	Likely	Abnormal
20% sugar	Cane sugar	0.21032	Likely	Abnormal

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

011963_01 PKI

APPLICATION BRIEF

FT-IR NIR Spectrometry

Author:

Ian Robertson

PerkinElmer, Inc.
Shelton, CT

Single NIR Measurement for the Detection of Adulteration and Measurement of Important Parameters in Cocoa Powders

Introduction

Cocoa powder is a product regularly used within the personal care, food, and beverages sectors. There have been reports indicating several health benefits of cocoa, from lowering blood pressure antioxidants to containing essential fatty acids, fiber, and minerals.

There have been cases of cocoa adulteration reported in Eastern Europe and across the world. In a recent case, samples contained 20% less cocoa compared to values listed on the labels, which was found to be fraudulent activity by the supplier.[1]

The initial method used to assess the quality of cocoa beans is the cut-and-taste test, although this is a subjective technique. Liquid chromatography methods are also often used for testing cocoa powder, but they are time consuming and can be complicated. A much faster and easier technique to verify the authenticity of cocoa is Near-Infrared (NIR) spectroscopy.

PerkinElmer®
For the Better

NIR Methodology

Spectra of six cocoa brands and two adulterants (arrowroot and dark rye flour) were collected on a PerkinElmer Frontier™ NIR spectrometer in reflectance using the NIRA II sampling accessory at a spectral resolution of 16 cm^{-1} using 32 scans. These spectra were entered into PerkinElmer's Adulterant Screen™ in the Spectrum 10 software as a library of material (good) and adulterant spectra, as shown in Figure 1. Different lots of one of the cocoa samples were then spiked at concentrations of 10% and 20% weight/weight concentration of arrow root and dark rye flour to test the capability of Adulterant Screen™.

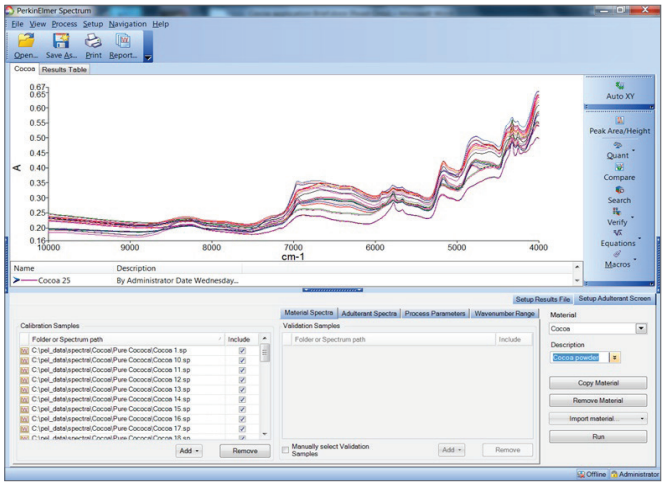


Figure 1. Adulterant Screen setup for cocoa powder showing cocoa powder material spectra.

Adulterants are commonly added to products at for financial gain. Adulterant Screen is able to correctly identify the adulterant used and estimate its concentration, as shown in Table 1, and can give an estimated level of detection of the adulterant, in this case <1%.

Table 1. Results from Adulterant Screen for spiked samples.

Sample Name	Adulterant	Level	Confidence	Material Fit
10% Dark Rye	Dark Rye Wholemeal Flour	0.10111	Likely	Abnormal
20% Dark Rye	Dark Rye Wholemeal Flour	0.18440	Likely	Abnormal
10% Arrow	Arrowroot	0.10105	Likely	Abnormal
20% Arrow	Arrowroot	0.17882	Likely	Abnormal

Adulterant Screen can be deployed using Spectrum Touch™ methods. This allows for an easy-to-use software environment for routine operators. A sample spiked with 1% Dark Rye Wholemeal Flour was tested using a Spectrum Touch method and the same Adulterant Screen. The resulting output in Figure 2 shows that the sample failed because adulterants were detected.

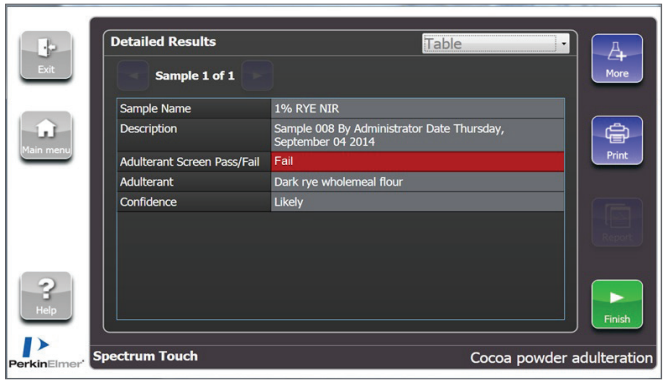


Figure 2. Spectrum Touch method deployment for an adulterated cocoa sample.

The NIR spectra of the samples also have the capability of determining the fat and dry-mass contents of cocoa samples. Cocoa 1 and 6 contained 22.6 g and 2.3 g of fat per 100 g of cocoa powder high-fat, respectively. The different fat levels are apparent in the spectra shown in Figure 3, particularly in the first overtone region of the C-H stretch, just below 5,900 cm^{-1} and the combination region at about 4,300 cm^{-1}.

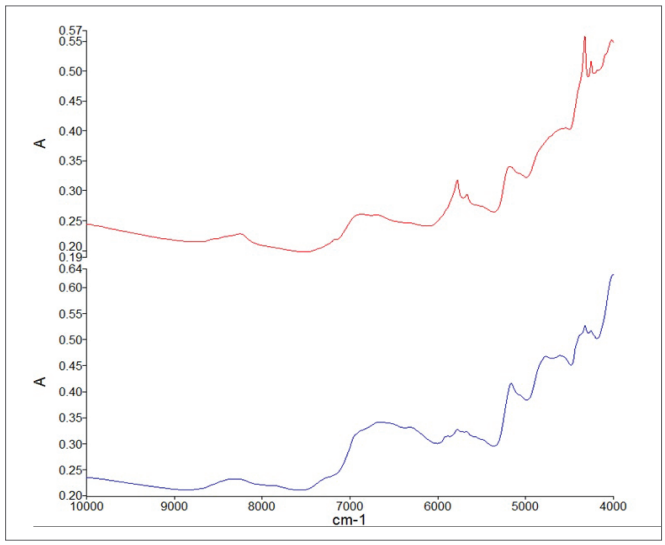


Figure 3. NIR spectra of high fat cocoa (top) and low-fat cocoa (bottom).

The spectral differences can be further highlighted by applying the second derivative to the spectra as shown in Figure 4. Applying the second derivative to the spectra will remove any broad baseline slope and offset due to the different scattering properties of the powders. These spectra show clear differences between the low- and high-fat cocoa powders and would form the basis of any quantitative measurement.

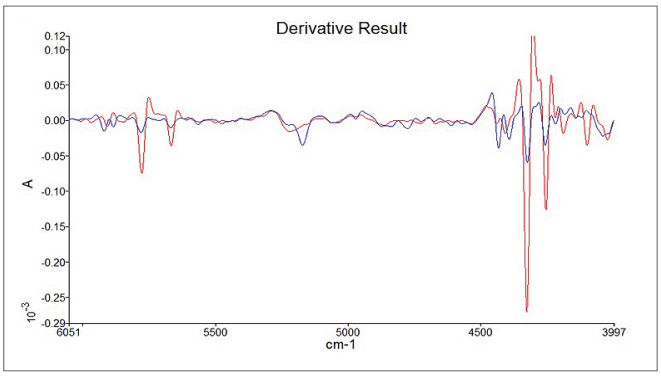


Figure 4. 2nd derivative of high-fat cocoa (red) low-fat cocoa (blue).

The dry-mass content of the powders can be measured in the combination region of the spectrum just above 5200 cm^{-1}. The second derivative spectra of a series of cocoa powders with very small variation in the dry-mass content are shown in Figure 5.

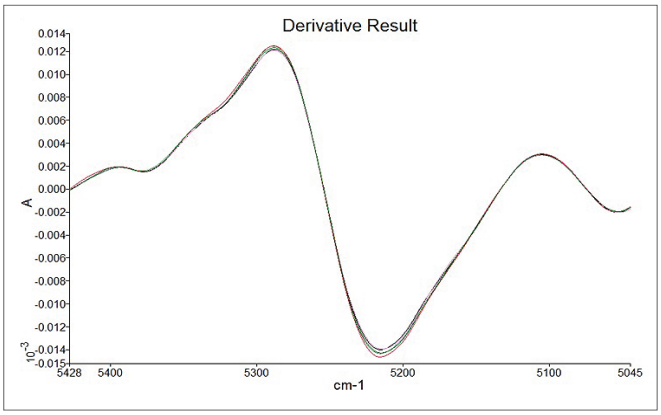


Figure 5. Variation in dry-mass content of cocoa powders.

This spectral region would form the basis for a quantitative measurement of the dry-mass content of the cocoa powders.

Conclusion

A single NIR measurement of cocoa powders can allow easy measurements for adulteration of the material and, with calibrations, also allow for determination of fat and dry-mass content within cocoa powder. Adulterant Screen allows for a fast, easy, and low-cost method for screening adulterants within cocoa powder. New adulterants can be added into the method simply by measuring the spectrum of the pure adulterant. This results in an easier method for the detection of adulteration in cocoa powder.

Reference

1. Czech Agriculture and Food Inspection Authority: Press Release Polish cocoa adulteration classes in Kaufland 04/25/2012

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

011957_01 PKI

APPLICATION NOTE

FT-IR Spectroscopy

Author:

Justin Lang, PhD

Lauren McNitt

PerkinElmer, Inc.
Shelton, CT

The Use of FT-IR Spectroscopy as a Technique for Verifying Maple Syrup Authenticity

Introduction

Although usually not thought of until pancakes or waffles are on the table, maple syrup is a serious business. It is one of the key crops where demand is greater than supply. Surprisingly, it takes 10 gallons of sugar maple tree sap to produce one quart of maple syrup. Because the syrup produced is only 1/40th of the actual sap yield, unscrupulous syrup suppliers are tempted to fraudulently adulterate their products with lower value commodities, in order to maximize their profit. Adulterants include cane syrup, high fructose corn syrup, beet syrup, and rice syrup. Infrared spectroscopy is shown here to be a fast and easy technique for detection and identification of these adulterants.

PerkinElmer®
For the Better

Method

Samples of Grade A maple syrup, corn syrup, high fructose corn syrup, and rice syrup were analyzed on the PerkinElmer Frontier™ Fourier Transform Infrared (FT-IR) spectrometer from 4,000 to 650 cm^{-1}, using a three-bounce Universal Attenuated Total Reflectance (UATR) sampling accessory. Samples were scanned by placing a single drop directly onto the diamond crystal of the UATR. After scanning, the UATR was cleaned using isopropyl alcohol on a laboratory wipe. Seven replicate measurements were performed for each sample type using a fresh aliquot for each scanned sample. Additional dilutions of maple syrup with the adulterants were prepared and scanned in order to validate the method.

Spectra of maple syrup and two of the common adulterants are shown in Figure 1A and an expanded region of interest in Figure 1B.

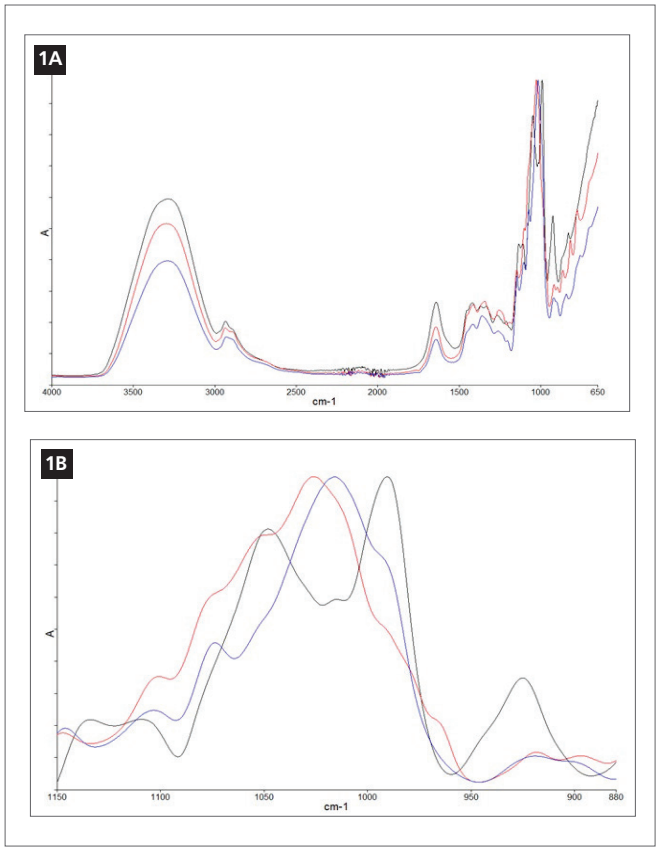


Figure 1A. FT-IR overlay of maple syrup and its common adulterants. maple syrup (black), high fructose corn syrup (red), rice syrup (blue). *Figure 1B.* Expanded spectral overlay of maple syrup (black), rice syrup (blue), and high fructose corn syrup (red), from 1150-880 cm^{-1}.

The spectra of these materials exhibit differences particularly in the spectral region from 1100 - 900 cm^{-1}.

A Soft Independent Model by Class Analogy (SIMCA) model was created to see if there was a measureable difference between the maple syrup and the adulterants. The SIMCA model is shown as Figure 2.

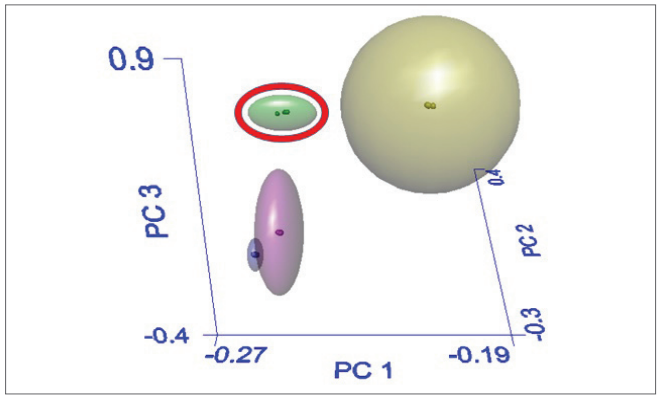


Figure 2. SIMCA model for the maple syrup and adulterants dataset (maple syrup highlighted).

There is good separation between maple syrup and the other adulterants, with a little overlap between the corn syrup and rice syrup. This model could be used to determine if the sample of interest is a maple syrup or not.

An Adulterant Screen™ method was set up using all of the maple syrup spectra as "Material Spectra" and single spectra of the adulterants as the "Adulterant Spectra". A Spectrum Touch™ method was set up incorporating the SIMCA model and the Adulterant Screen method into a simple user interface for the routine analyst. The model and method were tested using one of the diluted samples, 10% high-fructose corn syrup in maple syrup. The Spectrum Touch results screen is shown as Figure 3.

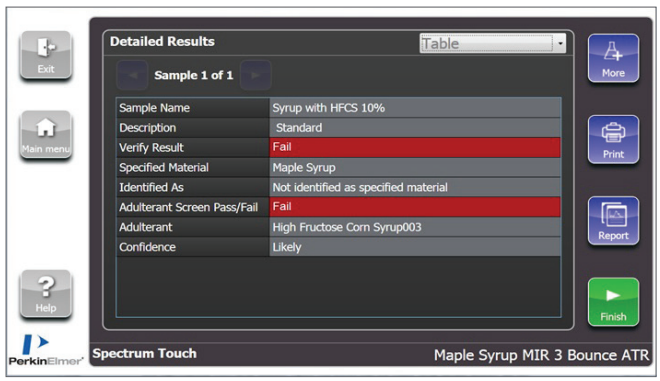


Figure 3. Spectrum Touch results screen.

This test sample fails both the SIMCA and the Adulterant Screen analysis. SIMCA indicates that this test sample does not conform to the maple syrup spectra in the model and Adulterant Screen states that adulteration is likely with high fructose corn syrup.

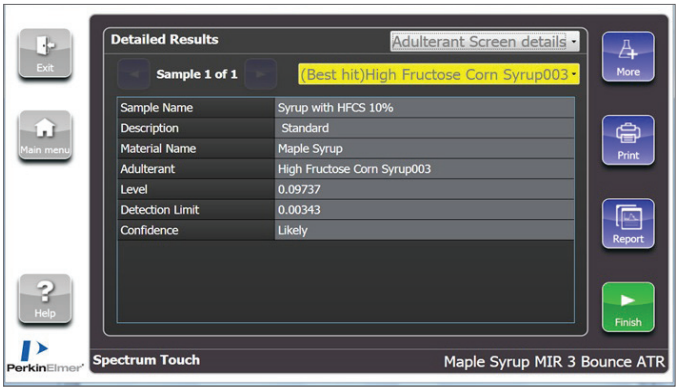


Figure 4. Spectrum Touch results screen highlighting the "Adulterant Screen details" view.

The Adulterant Screen results not only predict which adulterant is present, it will also predict how much of that adulterant is present and estimate its detection limit. This is achieved without the need for the lengthy process of preparing and measuring spectra of calibration standards. In this case, Adulterant Screen predicts a concentration level for the high-fructose corn syrup adulterant at 9.737%, very close to the actual concentration of 10%.

Conclusion

As maple syrup is a prime target for food fraud, there is a clear need to test for its authenticity. It has been demonstrated that utilizing an FT-IR empowered method with Adulterant Screen and SIMCA allows for the measurement of maple syrup quality and detects any adulterants that may be present. The advantage of Adulterant Screen is that it only requires base materials for the method and is fast and easy to use. This dramatically reduces the time required to develop a screening method as quantitative calibration development is not required. Additional adulterants can readily be added into Adulterant Screen without having to recalibrate the method.

References

- http://www.mi-maplesyrup.com/education/facts.htm
- http://tapmytrees.com/coprsap.html

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

011961_01 PKI

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

014336_01 PKI