Molecular Spectroscopy Compendium

ENSURE FOOD QUALITY, PRODUCTION, AND SAFETY

The Measure of Confidence

Agilent Technologies

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In this compendium, you'll find current and emerging applications that will help you identify both target and non-target molecules by applying the very latest techniques for spectral data gathering.

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IDENTIFY TARGETS AND UNKNOWNS IN FOOD MATRICES

Widespread globalization has increased the pressure to meet stringent standards for quality, safety, and cost effectiveness throughout the food production chain – including incoming inspection, new product development, and packaging.

FTIR, UV-Vis, and fluorescence spectrometers deliver sensitive, productive analyses from farm to fork

Short analysis times, limited sample preparation, and the ability to determine molecular structure make molecular spectroscopy ideal for many food analysis tasks:

Authenticating ingredients

- Verify identity by searching spectral databases
- Measure authenticity and alcohol content in spirits, liquors, and wine
- Confirm quality and authenticity of edible oils, syrups, and dairy products

Ensuring product quality

- Make sure food coloration is within specification
- Perform critical measurements, such as:
  - Antioxidant and vitamin levels
  - Levels of trans fats and free fatty acids
  - Quality traits in vegetables and fruits
  - Oxidation and its relationship to rancidity
  - Levels of acrylamide, and other unwanted by-products of food processing
- Research and control the quality of packaging materials
- Identify extraneous micro-contaminants and particles

(Continued)

Enhancing crop production

- Measure fruit ripeness to confirm that prices are based on quality
- Analyze soils and soil composition
- Identify pesticides in containers *before* distribution and application

As the food industry's premier measurement company, Agilent is uniquely positioned to help you protect lives and livelihoods. Our chromatography and mass spectroscopy systems are used in thousands of food analysis laboratories worldwide. We also support your food testing efforts with an extensive support network, plus a wide array of columns and supplies.

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Get precise, accurate answers with Agilent Molecular Spectroscopy instruments

New research, production, and quality-control applications for FTIR, UV-Vis, and fluorescence spectrometers are appearing every day. Agilent spectrometers have the power and versatility to handle the most challenging applications in the lab, on the farm, or at collection, shipping, and receiving sites.

Superior quantitative and qualitative data with excellent low-level sensitivity

Leading-edge Agilent molecular spectrometers enable researchers, suppliers, and producers to:

- Rapidly identify, verify, and authenticate food ingredients
- Quickly screen a representative number of incoming ingredients using portable FTIR systems – and make accept-or-reject decisions on the spot
- Ensure that incorrectly labeled, substandard, or unacceptable ingredients never enter the manufacturing chain
- Detect, identify, and measure contaminants in food and food ingredients
- Ensure the quality and performance of packaging materials
- Determine crop health and produce quality

In addition, Agilent Molecular Spectroscopy solutions are well matched to the food industry's need for speed, simplicity, and reliable answers.

- **Ultra compact systems** preserve valuable bench space, while making portable and mobile operation a reality
- **Mid-infrared FTIR spectrometers** provide more specific details about food sample composition, as compared to near-infrared (NIR) spectroscopy
- **Handheld and portable systems** let you bring your analyzer to your samples – in field, farm, collection, shipping, receiving, or processing locations
- **Benchtop and mobile FTIR systems** use the same optomechanical and electronic components – as well as the same sampling technology – simplifying method transfer from lab to field
- **Intuitive software** automates on-board methods, and enables any user to achieve reliable results
- **Exclusive FTIR sampling technology,** such as DialPath transmission interface, makes analyzing foods faster, more reliable, and easier than ever

AGILENT FTIR INSTRUMENTS

The Agilent FTIR range is unrivaled, sensitive and flexible. From benchtop instruments offering the highest spectral resolution and signal-to-noise (S/N) performance to dedicated portable analyzers, there is an instrument to suit every need.

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The 4100 ExoScan, 4500, 5500, and Cary 630 FTIR are all built on the same compact, robust technology and utilize the same software design. This ensures you can take quality measurements wherever you need them – simply choose the model that best suits your application.

In the lab or in the field
4100 ExoScan Series FTIR (handheld)

Our rugged, versatile 4100 ExoScan FTIR lets you screen for key analytes virtually anywhere, or bring samples back to the lab for more in-depth analysis. You can also choose from several sample interfaces that can be changed in seconds with *no realignment*. The system is ideal for measuring a wide range of foods, food ingredients, and agriculture-related substances – such as soils, fertilizers, herbicides, and pesticides – often with no sample preparation required.

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Reliable, rugged, and easy to use
4500 Series Portable FTIR

Agilent 4500 Series FTIR spectrometers are fully portable, battery-powered analyzers suitable for testing foods and food ingredients in demanding environments – such as agricultural fields, produce collection sites, and raw ingredient shipping/receiving sites. The 4500 series FTIR is also well-suited for rapidly verifying identity and quality.

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(Continued)

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High-quality answers in less than one minute
5500 Series Compact FTIR

From intuitive software... to "one button" operation... to rapid execution of on-board methods... Agilent 5500 Series FTIR Spectrometers enable rapid screening and quality analysis of incoming foods and ingredients. Their ultra-compact design also makes them perfect for at-site labs and mobile vans.

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Confident analysis and method development
Cary 630 FTIR Spectrometer

The ultra-compact Agilent Cary 630 FTIR delivers superior quantitative and qualitative data for routine analysis of solids, semi-solids, and liquids. It offers a wide selection of interchangeable sampling accessories, such as our exclusive DialPath technology for liquid samples and diamond ATR for solids and semi-solids.

In addition, the optics of the Agilent Cary 630 are nearly identical to those of other Agilent portable and handheld FTIR spectrometers. That means you can use the system as a method development platform to support in-field analyses closer to the sample location.

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Extend your FTIR spectrometer's capabilities
Cary 610 FTIR Microscope

Combining the Cary 610 FTIR microscope with the Cary 660 FTIR creates an ideal platform for analyzing small and heterogeneous samples. You can also choose between transmission, reflection, ATR, and grazing angle measurement modes to support your analysis of microcontaminants, and your development/quality control of polymer laminate food packaging.

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FTIR Application Notes

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Fast, effective evaluation of edible bird nests using the handheld Agilent 4100 ExoScan FTIR

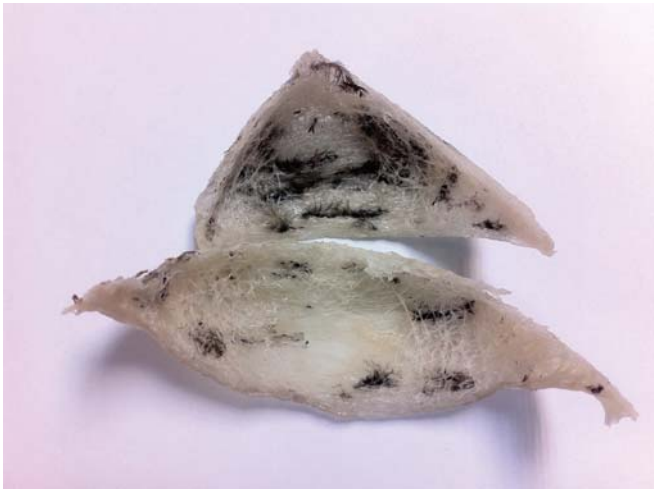
Application note

Food testing

Author

Joe Set

Agilent Technologies
Petaling Jaya, Malaysia



Introduction

The popularity of birds nest soup in Chinese cuisine stretches back over 1000 years. The literal translation of the Chinese name for the dish, yàn wō (燕窝), is "swallow's nest". In fact, edible bird nests are produced by swiftlets (Collocalia fuciphaga), which belong to the same family as the common swallow, but which are smaller in size. Although there are 90 varieties of Southeast Asian swiftlet, the nests of only four types of bird, namely cave swifts, are deemed worthy of human consumption. During the breeding season, the male birds regurgitate a sticky salivary secretion to form the nest. As a rich source of amino acids, carbohydrates and mineral salts, bird nests have also been used for hundreds of years as an important health supplement in Traditional Chinese Medicines (TCM). Examples of its use include as a treatment for malnutrition, a boost to the immune system, and to enhance the body's metabolism. More recently bird nests have also been used in cosmetic products.

The high demand for edible bird nests, especially from China, Hong Kong and Taiwan, and limited supply has led to a lucrative market building up around the product. This in turn has led to the upsurge of fake and adulterated edible bird nest products. Unethical suppliers blend the original bird nest with additives in order to boost its weight and market value. Common substances used to imitate bird nests include edible plants, fish skin, mushroom or algae [1]. The more expensive 'Red Blood' bird nest can be adulterated from the less expensive white nest by adding a natural colorant such as karayagum, red seaweed or tremella fungus or by exposing it to sodium nitrate.

The method that is required to identify genuine, unadulterated bird nests also needs to be applied to the food safety arising from the production and preparation of nests for consumption. Edible bird nests undergo treatment with hydrogen peroxide to 'whiten' the nests. Many processes involve the addition of preservatives such as boric acid, potassium sulfite or sulfur dioxide (according to country regulations). Sugar, salt, and monosodium glutamate (MSG) are added to improve the taste. Gluten, white fungus, jelly, animal skin or synthetic rubber is often used to improve the shape and appearance of the nests.

This application note provides a simple method for the authentication of edible bird nests, the identification of adulterants and food safety using handheld Fourier transform infrared (FTIR) spectroscopy. IR spectroscopy measures the covalent chemical bonds, creating a molecular 'fingerprint' of the chemicals present. This fingerprint can be used to identify and quantify chemicals present in a sample. FTIR spectroscopy is commonly used for the identification and contamination of food products. An example of edible bird nest analysis by conventional FTIR is shown in Figure 1. In this example, major bands can be observed resulting from protein at 1640 and 1550 cm^{-1} , carbohydrate near 1030 cm^{-1}, and lipids near 2930 cm^{-1}. Clear differences can be seen between the clean and adulterated samples. Recent advancements have reduced the size and weight of FTIR spectrometers, resulting in easy to use handheld systems. This new technology now allows for non-destructive analysis, which is particularly useful for expensive food products such as edible bird nests.

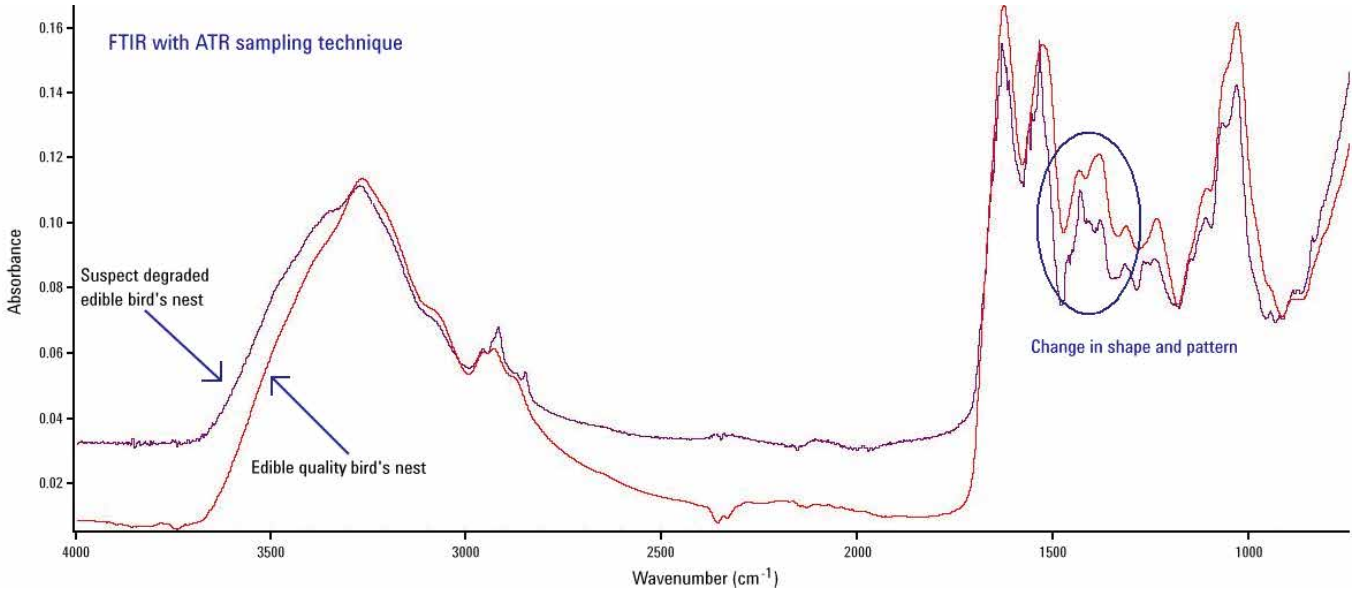


Figure 1. Infrared spectra of a pure edible bird nest and an adulterated edible bird nest acquired using a bench FTIR spectrometer with attenuated total reflectance (ATR)

Instrumentation

An Agilent 4100 ExoScan FTIR (Figure 2) was used for this study. It is a versatile, robust handheld mid-IR system that can be equipped with a wide range of interchangeable sampling interfaces. In this study, the diffuse reflectance interface was used. The 4100 ExoScan FTIR is powered by rechargeable and interchangeable lithium ion batteries that can be operated continuously for 4 hours. It can also be docked in the lab and operated like a benchtop FTIR, with performance equal to conventional laboratory FTIR spectrometers.

Used with the diffuse reflectance sample interface, the 4100 ExoScan FTIR provides non-destructive testing. This is in contrast to the a more conventional lab benchtop FTIR using an attenuated total reflectance (ATR) sampling method that would destroy the physical structure of a bird nest. Additionally, the diffuse reflectance sample interface measures a greater sample volume, yielding a more sensitive measurement than ATR. In addition to providing non-destructive testing, the compact design of the 4100 ExoScan means that sample analysis can take place where it is most convenient, whether in a lab, mobile facility or in the field.

Diffuse re ectance mid IR spectroscopy of edible bird nests

Diffuse reflectance IR spectra of neat samples have a different band shape compared to classical absorbance spectra as shown in Figure 3. Highly absorbing samples produce negative bands when plotted on an absorbance scale. These negative bands are due to changes in the refractive index of the material coincident with the absorbance band. The bands are, however, at the same frequency as positive absorbance bands measured with ATR and can be used to identify and quantify materials in a similar manner. Although the band shape is different, the information content is the same between measurements made with diffuse reflectance and ATR sample interfaces.

As was stated above, edible bird nests can be contaminated or adulterated with a number of common additives including carbonates, sugars, salt, MSG and feathers. Examples of adulterated bird nests were measured with the 4100 ExoScan FTIR. Spectra obtained from these adulterated samples are shown in Figures 4 to 7, demonstrating the ease of detection. Furthermore, a method was created to give a pass/fail answer, and identify the adulterant as detailed in the following section.

Figure 2. The Agilent 4100 ExoScan FTIR (left) and in use (right), testing an edible bird nest

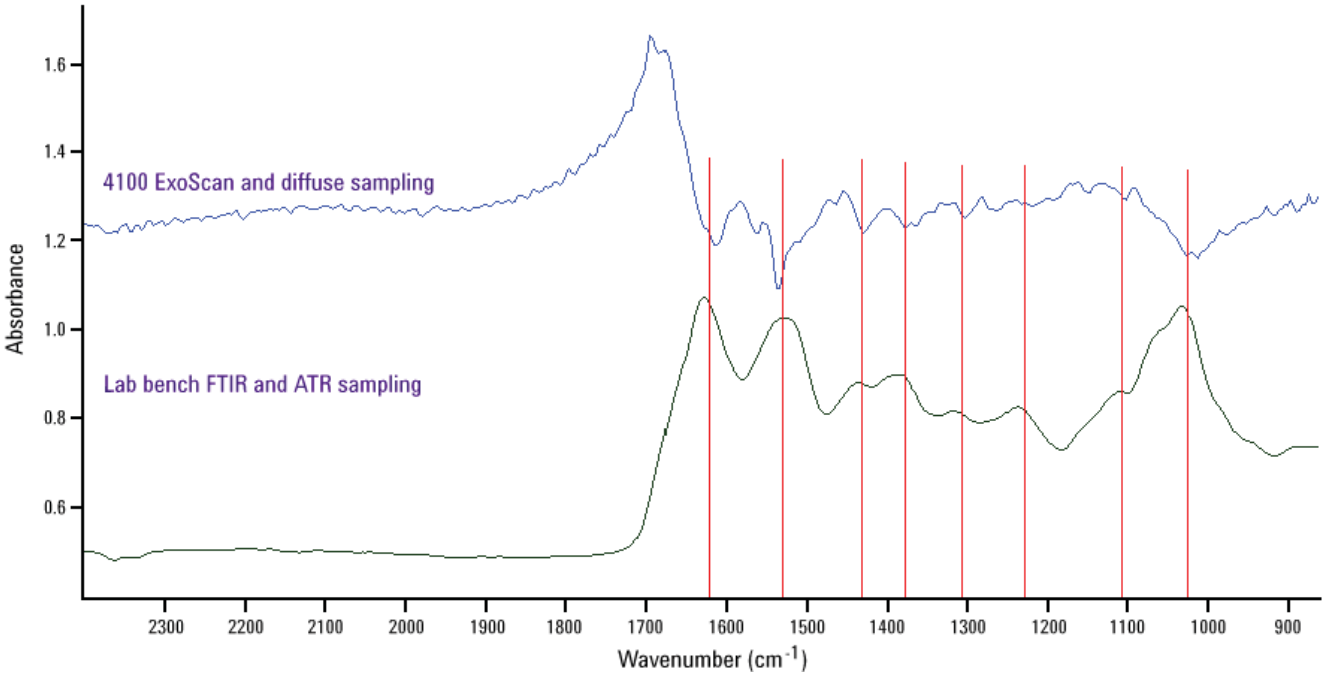


Figure 3. Lab bench FTIR with ATR sampling compared to 4100 ExoScan with diffuse sampling. The identical sample has been measured by both sampling techniques.

Figure 4 shows a comparison of a spectrum obtained from a pure edible bird nest sample, and one that has been contaminated with calcium carbonate, most likely arising from the nest's original location on limestone rocks or cave. The pure nest is characterized by spectral bands due to the protein (amide I, II and III) as well as bands due to cellulose. In addition to those bands, the nest contaminated with calcium carbonate contains two additional bands at 1410 cm^{-1} and 873 cm^{-1} due to the carbonate functional group. As can be seen from the spectra in Figure 4, the 4100 ExoScan FTIR can easily distinguish a pure edible bird nest sample from one that has been contaminated with calcium carbonate.

Salt (sodium chloride) is often added to an edible bird nest to enhance its flavor and to increase its weight and value. Sodium chloride is an inorganic compound; therefore, it is not visible by IR. However, adulteration of edible bird nests by salt is still detectable with the 4100 ExoScan FTIR. Salt effectively 'dilutes' the sample, causing the spectrum to exhibit positive bands where a spectrum of pure bird nest has negative bands. This can be seen in Figure 5.

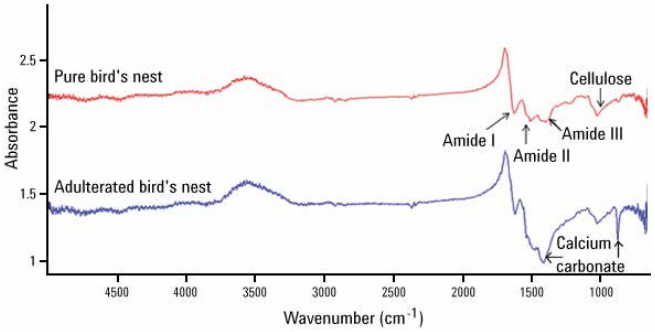


Figure 4. Identification of calcium carbonate contamination of a bird's nest measured with the Agilent 4100 ExoScan FTIR in diffuse sampling mode

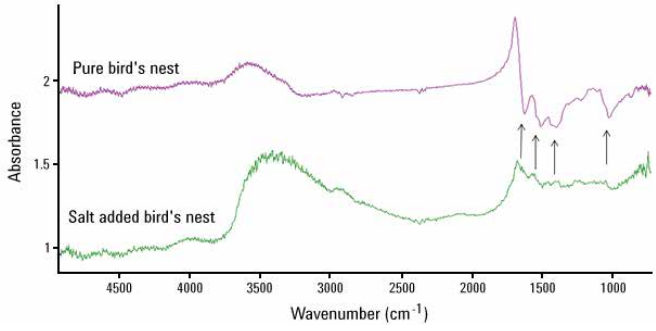


Figure 5. Identification of salt present in bird's nest measured with the Agilent 4100 ExoScan FTIR in diffuse sampling mode

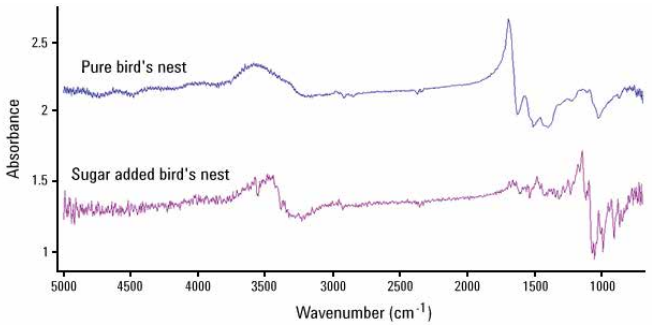


Figure 6a. Clear identification of sugar (sucrose) present in an edible bird nest measured with the Agilent 4100 ExoScan FTIR in diffuse sampling mode

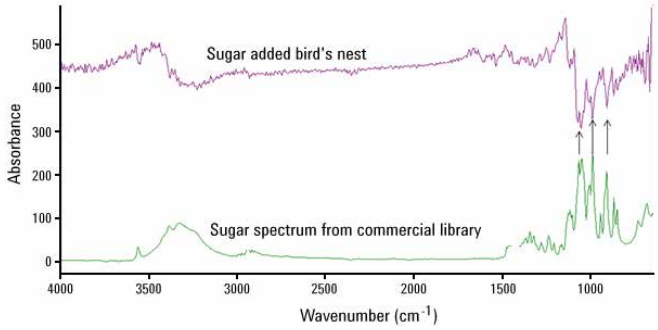


Figure 6b. Comparison of a commercial library ATR spectrum of sucrose and the Agilent 4100 ExoScan FTIR spectrum of sugar added to a bird's nest

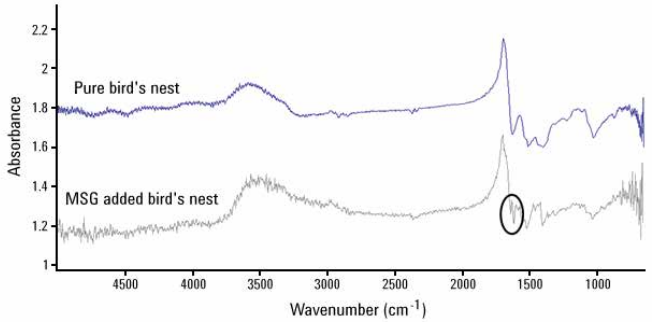


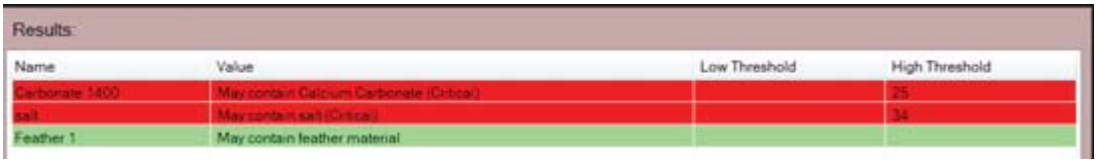
Figure 7. Identification of MSG added to an edible bird's nest measured with the Agilent 4100 ExoScan FTIR in diffuse sampling mode

Like salt, table sugar (sucrose) can also be added to a bird nest to increase its weight and improve its taste. Sucrose is easily identifiable in edible bird nests using FTIR as shown in Figure 6a. The spectrum of the adulterated sample shows strong sucrose bands at 1050 cm^{-1}, 980 cm^{-1} and 905 cm^{-1}. This is confirmed by reference to a commercial library ATR spectrum of sucrose (Figure 6b).

Mono sodium glutamate (MSG) is another common food additive that is added to edible bird nests as a flavor enhancer. As shown in Figure 7, edible bird nests adulterated by MSG can be identified by a doublet that overlaps with the protein Amide I band corresponding to the glutamate, which is the salt of a carboxylic acid.

Pass/Fail Methodology

The results show that many types of common adulterations can be observed in the infrared spectrum of edible bird nests. In order to provide a conclusive answer for inspectors, a unique method was developed that gives both a pass/fail result and the identity of common contaminants. This method is implemented in the Agilent MicroLab Mobile FTIR software. MicroLab Mobile operates the 4100 ExoScan FTIR, and is designed to provide easy to interpret qualitative or quantitative results. In this case a quantitative component was defined for each adulterant. If the measured component is above a predefined threshold, the adulterant is displayed in red. This gives the user an easy to interpret indication that the sample is adulterated, and the identity of the adulterant. Figure 8 shows an example of a bird's nest adulterated with both carbonate and salt. Also included is a measurement of the residual feather material, which reduces the value of a bird nest. This method is superior to more common library search or correlation methods because it can detect small quantities of adulterants that would be invisible to most library search methods.


Results:

Name	Value	Low Threshold	High Threshold
Carbonate 1400	May contain Calcium Carbonate (Critical)		25
salt	May contain salt (Critical)		34
Feather 1	May contain feather material		

Figure 8. Agilent 4100 ExoScan FTIR results screen showing additives and contamination of a sample of an edible bird nest

Conclusions

FTIR spectroscopy is a valuable technique for the identification, quality assurance and authentication of edible bird nests as it is non-destructive, fast and direct, requiring no sample preparation, with good sensitivity and specificity. These attributes are important to help prevent the fraudulent trade of counterfeit or adulterated bird nests and also to assure food safety and health standards. The industry surrounding the supply, processing and selling of swiftlet nests is expanding rapidly due to a growing affluence in China, the largest market for edible bird nests, and publicity surrounding the health and well-being benefits of consuming bird nests.

The Agilent 4100 ExoScan FTIR analyzer fitted with an integrated diffuse reflectance sampling interface provides an ideal analytical capability for the application:

- It is easy to operate using a Select-Point-Press mode, making it ideal for unskilled operators.
- Samples can be analyzed in the field using the light and portable analyzer ensuring quick results and fast sample turnaround, allowing adulterated samples to be rejected at source.
- A number of areas on the sample can be analyzed in a non-destructive manner allowing each nest to be tested.
- The results are easy to interpret using the unique quantitative method available with the MicroLab Mobile software.
- The results generated are reliable, as illustrated by comparison with data acquired using conventional technology.

References

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Agilent Technologies

In situ screening of quality traits in tomato cultivars using the handheld 4200 FlexScan FTIR spectrometer

Application note

Food testing

Authors

Glynda Fe G. Sayajon*, Sarah Herringshaw*, Alejandra M. Santos*, Luis E. Rodriguez-Saona* and David M. Francis†

The Ohio State University
* Columbus, † Wooster, OH, USA

Abstract

Chemometrics was employed to develop models that utilize infrared spectral measurements to estimate the concentrations of key analytes in tomatoes that determine quality. Models constructed using the handheld Agilent 4200 FlexScan FTIR spectrometer gave results comparable to those obtained using a benchtop spectrometer. The 4200 FlexScan spectrometer thus provides the ability to accurately assess the quality of tomatoes in the field in order to assure the quality of the end food product.

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Introduction

Monitoring the quality of tomatoes (*Lycopersicon esculentum*) is important at all levels in the food industry. While appearance, texture and flavor are the important quality attributes of fresh-market tomatoes, the major quality components of processing tomatoes are soluble solids, pH, titratable acidity, viscosity and color.[1] Sugars and organic acids, responsible for the sweetness and tartness, are the major factors affecting consumer acceptability[2] and the sugar-acid ratio is vital to flavor acceptability.[3] In the tomato industry, soluble solids dictate the factory yield: the higher the soluble solids, the lesser the quantity of tomato that is required to produce processed tomato products such as pulp, paste and concentrate[4]. Similarly, pH plays a vital part in microbiological safety and food spoilage. Generally, the pH of tomatoes has been reported to range from 3.9 to 4.9, or in standard cultivars, 4.0 to 4.75.

The development of tomato varieties with altered nutritional profiles requires efficient selection and scientific competence to measure metabolite contents in thousands of samples[6]. Field-based devices can streamline quality assurance and Fourier-transform infrared (FTIR) techniques combined with chemometrics offer tomato processors and breeders powerful tools for the rapid assessment of tomato quality attributes. Portable IR units enable the food manufacturer to quickly assess the quality of the product, allowing for timely correction measures during manufacture. Portable systems are simple to use and require minimal or no sample preparation. They reduce assay time and help to streamline the analytical procedure so that it is more applicable to higher sample throughput and automation, providing in-situ assessment of the sample's composition.

This application note evaluates the performance of the novel Agilent 4200 FlexScan portable infrared units against a benchtop IR spectrometer for the determination of tomato quality parameters (Brix, pH, titratable acidity, glucose, fructose and citric acid). Models were constructed on both spectrometers using partial least squares regression (PLSR) to predict these quality parameters, using reference data collected by traditional refractometer and chromatography methods. The 4200 FlexScan demonstrated comparable performance to the benchtop spectrometer for the measurement of these key quality parameters, with the added advantage of delivering that information to food scientists in the field, providing increased efficiency and efficacy in assuring the quality of the end food product.

Experimental

Samples

A total of 80 tomato varieties (genotypes) was grown in Fremont, Ohio at the Ohio Agricultural Research and Development Center (OARDC), North Central Agricultural Experimental Station in 2009. The varieties represent landrace varieties from Central and South America, and vintage varieties from North America and Europe.

At least 12 representative fruit from each variety were cut into quarters and a quarter from each fruit was pooled and blended into juice.

Reference measurements

Soluble solid content was measured using an Abbe refractometer. The overall pH of diluted samples of puree were measured and the samples were titrated to pH 8.2 using 0.1N NaOH to determine titratable acidity. This was reported as % citric acid using the conversion factor of 0.064 Equivalent wt. (mg/mEq).

Sugar and acid contents were analyzed using high performance liquid chromatography. For extraction of sugars, tomato juice aliquots (~2 mL) were passed through a mixed-mode C18 and strong cation exchange SPE column to remove interfering acidic components from the solution. For extraction of acids, aliquots of tomato juice (2 mL) were mixed with 2% metaphosphoric acid and centrifuged.

Infrared measurements

Spectral data from all tomato juice samples were collected in parallel using (1) a dry air purged benchtop Excalibur Series 3100 Fourier-Transform infrared spectrometer (Varian, now Agilent Technologies, Santa Clara, CA) incorporating a KBr beamsplitter, DTGS detector, and three reflection ZnSe ATR accessory; (2) a handheld 4200 FlexScan FTIR spectrometer

(Agilent Technologies Inc., Danbury, CT), with a ZnSe beamsplitter, DTGS detector and single-reflection diamond ATR sampling interface. Both spectrometers were operated at 4 cm^{-1} spectral resolution.

Aliquots (0.5 mL) from thawed tomato juice samples were centrifuged at 12,000 rpm for 5 minutes at 25 °C, and two drops were applied to the surface of the ATR crystal for spectral data collection. The absorbance spectra of the room temperature samples consist of 64 co-added interferograms at 4 cm^{-1} resolution measured in the 4000 to 600 cm^{-1} mid-IR wavelength range. The spectrum of each individual sample, which took less than 2 minutes to acquire, was corrected against a background spectrum of air. Duplicate independent measurements were taken on each sample and background spectra were collected after every sample to account for environmental variations. Between measurements, the ATR surface was cleaned with a 70% ethanol solution.

Statistical analysis

Partial least squares regression (PLSR) analysis was used to correlate the infrared spectral results with those of the referee analysis methods. PLSR is a bilinear regression analysis method that decreases a huge number of variables into latent factors that are linear combinations of the spectral frequencies (X) used to ascertain the analyte's concentration (Y)[7]. This technique has the potential to estimate the component concentration, as well as the chemical properties of the spectra.[8] This analytical approach offers a more information-rich data set of reduced dimensionality and eliminates data noise, resulting in more accurate and reproducible calibration models[9].

Spectral data were exported from the spectrometers as .spc files and imported into a multivariate statistical program for partial least squares regression analysis in order to generate the calibration models. The calibration models correlated the spectra against the concentration of each tomato analyte (Brix, pH, titratable acids, glucose, fructose, and citric acid), which had been measured by the referee analysis method. Performance of these models was evaluated in terms of outlier diagnostics, standard error of cross-validation (SECV), and correlation coefficient (R). Distribution of residuals and leverage were used for the evaluation of outliers. Any observation with atypical and large residual or leverage was re-analyzed and eliminated if it was considered a substantial outlier, and thereafter the model was recalculated. The calibration model that generated the best combination of minimum SECV, higher R and optimized numbers of latent factors was selected for each tomato analyte.

Results and discussion

The spectra of 160 (80 varieties, 2 replicate spectra per variety) juice samples appeared quite homogeneous upon visual inspection, and no outliers were identified. The major absorbance bands at approximately 3300 and 1635 cm^{-1} (Figure 1A) arise from water in the samples and are consistent with those reported for other fruits and vegetables. The 1800–1000 cm^{-1} fingerprint region exhibited peaks corresponding to the carbonyl stretching groups (1726 cm^{-1}), C=C stretching of ring vibration (1605 cm^{-1}, 1509 cm^{-1}), O-H deformation (1365 cm^{-1}) and C-OH stretching (1262 cm^{-1}, 1145 cm^{-1}, 1035 cm^{-1}) associated to sugars and acids.

Second derivative mathematical transformation of the spectra (Figure 1B) helps to resolve overlapping bands and eliminates the need for baseline correction, since the most significant offset and linear baseline errors are removed.[10]

PLSR analysis was used to generate models that are linear combinations of these spectral frequencies that correlate to the concentrations of analytes in the tomatoes that were determined using the reference analysis methods. Models were constructed using the data from both the benchtop and the 4200 FlexScan spectrometers. These models were then used to determine the concentrations of these analytes in samples from a total of 80 tomato varieties.

By using varieties representing the landrace and vintage 'core collection' used to study genetic variation in cultivated tomato under the Solanaceae Coordinated Agricultural Project, a large compositional range was obtained with varieties showing elevated sugar and organic acid contents (Table 1). In general, the values reported in this study are within those reported in the

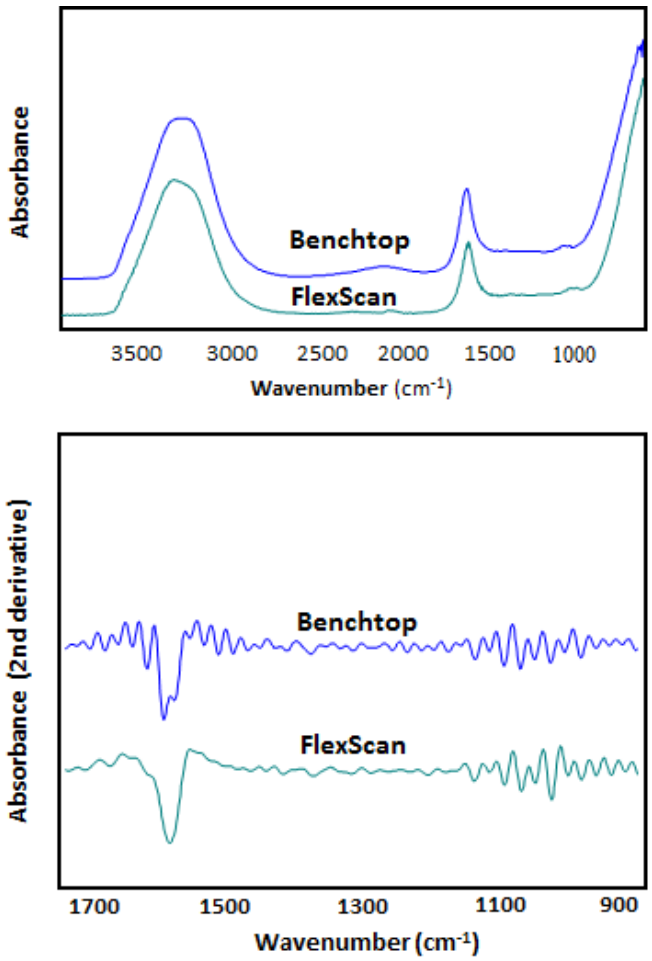

Figure 1. Attenuated total reflectance (ATR) infrared absorption spectrum of tomato sample (A) and its second derivative transformation (B) as shown on the benchtop Excalibur 3100 FTIR and portable Agilent 4200 FlexScan spectrometers

literature, taking into account that nutrient levels may be affected by variety, maturity, temperature and soil nutrients among others[11].

Since the benchtop spectrometer used a three-reflection ATR, it would follow that it would have higher sensitivity than the 4200 FlexScan handheld instrument equipped with a single reflection ATR. In spite of that and although the benchtop instrument provided the best quality parameter predictive models in general, the handheld 4200 FlexScan system generated very comparable correlation values (R) and SECVs (Table 1). The 4200 FlexScan handheld spectrometer can therefore provide reliable estimations of Brix, glucose, fructose, pH, titratable acids and citric acids in tomato samples

Table 1. Predictive models for determining tomato qualities by FTIR using a benchtop and the Agilent 4200 FlexScan spectrometers

Tomato analyte	Spectrometer	Concentration/ pH range	SECV	R
Brix	Benchtop	4.40–7.50	0.36	0.79
	FlexScan		0.39	0.75
Fructose	Benchtop	1.37–5.70 g/100 g	0.32	0.87
	FlexScan		0.43	0.79
Glucose	Benchtop	1.03–5.75 g/100 g	0.43	0.89
	FlexScan		0.49	0.86
pH	Benchtop	3.90–4.76	0.06	0.92
	FlexScan		0.06	0.91
Titratable acids	Benchtop	0.27–0.88	0.05	0.91
	FlexScan		0.05	0.87
Citric acid	Benchtop	256.37–2015.93 mg/100 g	136.50	0.90
	FlexScan		123.40	0.92

in the field. This information enables optimization of the quality of the end food product, since it can be obtained before harvest and processing.

The estimated analyte contents measured by the ATR-IR spectroscopy showed some data scattering along the regression line (Figure 2), when compared to those determined by the reference methods (refractometer, pH meter, or HPLC), as the R value ranged from 0.75–0.92 (Table 1). However, the Brix measurements had a ±0.1° accuracy, which were further complicated by rounding errors, and coefficients of variability (%CV) for HPLC analysis were ~20%, which could limit the precision of the PLSR prediction.

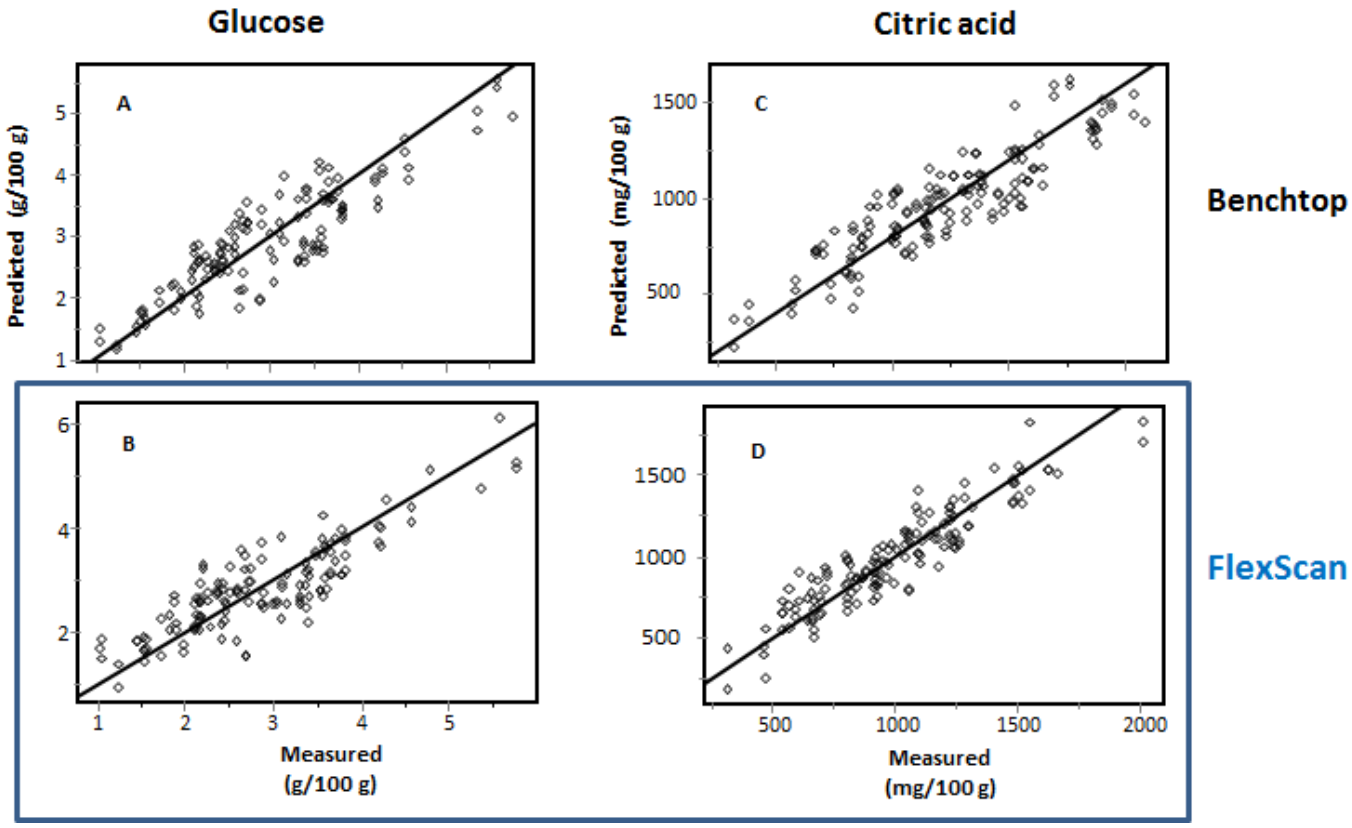

Figure 2. PLS regression plots of glucose (A and B) and citric acid (C and D) on the benchtop Excalibur 3100 FTIR (A and C) and 4200 FlexScan (B and D) spectrometers. The regression plots showed a good correlation per analyte across spectrometers.

Conclusions

Screening of quality parameters in tomatoes has been accomplished through the use of the handheld Agilent 4200 FlexScan FTIR spectrometer in the mid-infrared spectral region. A simple, quick and reliable technique was developed for the determination of key analytes and the results were comparable to those obtained with a benchtop unit. The ease of use, convenience and ruggedness offered by the handheld infrared spectrometer make it an ideal tool for food scientists and technicians to obtain vital information in the field. This tool can play a vital role in crop and process improvement studies, thus improving the quality assurance of the end food product.

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Agilent Technologies

QA/QC of dairy powders using the Agilent Cary 630 ATR-FTIR analyzer

Application note

Food testing and agriculture

Zubair Farooq and Ashraf A. Ismail

McGill University
McGill IR Group
Department of Food Science and Agricultural Chemistry
Montreal, Quebec
Canada

Introduction

Dairy powders are used extensively as functional ingredients in a myriad of food products. The composition of dairy powders can be highly variable in terms of proteins, carbohydrate (normally lactose), fat and moisture content. The characterization of dairy ingredients is traditionally carried out by solvent extraction of the fat, followed by chromatographic separation of the proteins and lactose. While these methods are highly accurate, they are time-consuming, tedious and costly. In recent years vibrational spectroscopy has been proven to be an ideal way to analyze powders, giving results within minutes of sample acquisition without prior sample preparation. Among the various vibrational techniques available: Fourier transform infrared (FTIR), near infrared (NIR), and Raman spectroscopy, mid-FTIR spectroscopy appears to be the most useful.

This is because mid-FTIR spectroscopy can provide a great deal of structural information from spectra characterized by very sharp spectral bands that can be assigned to specific functional groups, thereby making mid-FTIR spectroscopy a highly valuable technique for the identification of complex dairy ingredients. Furthermore, using an attenuated total reflectance (ATR) sampling accessory in combination with an FTIR spectrometer, as in the case of the Cary 630 ATR-FTIR analyzer, provides the best means for routine authentication of dairy powders and quality assurance/quality control (QA/QC) applications aimed at assessing the batch-to-batch variability of powder ingredients.

Objective

Government regulatory agencies and consumer groups are increasingly demanding that the food industry authenticate all raw materials going into their products. To address such a challenge in a timely and cost-effective manner, product manufacturers usually require an advance sample of food ingredients be shipped to them for testing prior to accepting a large quantity of the commodity. However, once the shipment arrives there is still the question of whether the advance samples were indeed representative of the bulk shipment and there is also a need to confirm that the bulk shipments are uniform in composition. For example, since most dairy powders to the naked eye appear white, with a soft grainy texture and a virtually identical structural consistency (Figure 1), it is necessary to utilize analytical methods to characterize their composition.

Figure 1. Various milk protein powders

Ideally, this analysis should be completed in less than 30 seconds, require little or no sample preparation and should be carried out at the receiving dock prior to placing the dairy powder ingredients into storage or integrating them directly into the production line.

In this application note, α-lactalbumin, an expensive whey protein, must be uniquely differentiated from other dairy ingredients such as β-lactoglobulin, whey protein isolate (WPI), whey protein concentrates (WPC) or even other types of dairy proteins such as caseins or caseinates.

Experimental

Materials and instrumentation

- Various milk protein powders obtained from different suppliers including α-lactalbumin, β-lactoglobulin, glycomacropeptide, milk protein concentrate, WPI, WPC, caseins and caseinates.
- A robust and light-weight Agilent Cary 630 ATR-FTIR analyzer (Figure 2).

Figure 2. Agilent Cary 630 ATR-FTIR analyzer

Method

Spectral acquisition was carried out by:

1. Placing a small amount of protein powder on the diamond ATR surface.

2. Pressing the samples against the diamond crystal using the attached pressure clamp. A slip clutch on the clamp prevents overtightening.

3. Collecting 64 co-added spectra (~30 seconds acquisition time at 4 cm^{-1} resolution) between 4000 and 650 cm^{-1}.

Data processing

A database of spectra of representative dairy powders was constructed using the Agilent MicroLab FTIR software spectral library builder. Subsequently, additional spectra of different lots of dairy powders were recorded and treated as 'unknowns'. For example, the ATR-FTIR spectrum of α-lactalbumin was acquired and treated as an 'unknown' and was identified correctly as α-lactalbumin through the automated spectral matching feature of the MicroLab FTIR software (Figure 3).

Figure 3. Correct identification of an unknown dairy powder as α-lactalbumin by the Agilent MicroLab FTIR software by direct comparison to a spectral library of dairy powders

Results and discussion

Figure 4 shows typical infrared spectra acquired using the Cary 630 ATR-FTIR analyzer for α-lactalbumin, β-lactoglobulin, WPI, and WPC. The difference between the other three proteins and WPC (attributed to the presence of lactose in WPC) can be clearly seen in the presence of additional bands between 1300 and 900 cm^{-1}. The differences among the three other proteins are not as easily distinguishable by the naked eye, but by utilizing the built-in spectral analysis MicroLab FTIR software, all four protein types can be immediately characterized and differentiated.

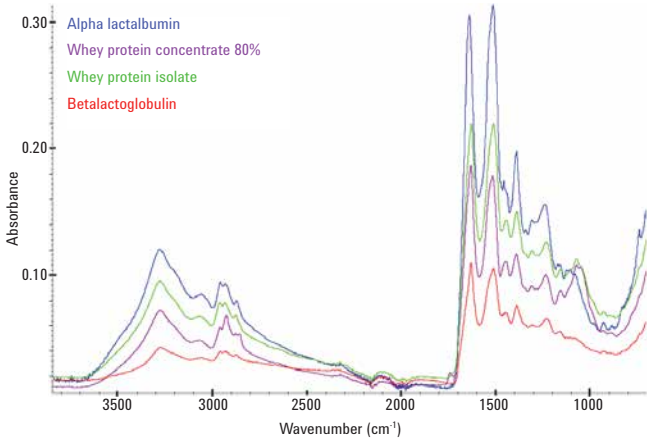

Figure 4. Infrared spectra of selected dairy powders recorded on the Cary 630 ATR-FTIR analyzer

Conclusion

The Agilent Cary 630 ATR-FTIR analyzer can dramatically decrease the cost and time for analysis of dairy powder ingredients with minimal training and virtually no sample preparation. The analyzer is compact, light-weight and portable making it ideal for use at the receiving dock, in production lines or in the QA/QC laboratory.

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Agilent Technologies

QA/QC of flours using the Agilent Cary 630 ATR-FTIR analyzer

Application note

Food testing and agriculture

Zubair Farooq and Ashraf A. Ismail

McGill University
McGill IR Group
Department of Food Science and Agricultural Chemistry
Montreal, Quebec
Canada

Introduction

Food grains (cereals, legumes and oilseeds) are milled to get flours, which can be employed in the manufacture of an enormous variety of products such as pasta and bakery products. All the raw ingredients in a food product should ideally be authenticated in order to ensure consistent quality standards and to comply with regulatory labeling requirements. Flours are generally received as powders that need to be tested to ensure: firstly that the contents of the containers correspond with the label and shipping ledger; and secondly, that the product received meets the requirements of the formulator as to uniformity and purity (in terms of composition). Typically an advance sample is made available before bulk shipment and, in this case, it is incumbent upon the formulator to verify that the advance sample is indistinguishable in composition from that of the bulk shipment.

Flours and flour products are usually composed of carbohydrates, proteins, lipids, moisture and other minor constituents. Flour is typically mixed with water to form dough, which is then used in the production of bread and other baked goods. Differentiation among the various flour types (for example, gluten-free flour) can ensure quality and address mandatory labeling requirements.

Experimental

Materials

- Various types of flours (Figure 1) such as chickpea, oat, rice, chestnut (gluten-free), millet (gluten-free), soya, yellow and white corn, organic shelled hemp seed (gluten-free), wheat gluten, whole wheat, breadcrumbs and baking soda were obtained from different suppliers.

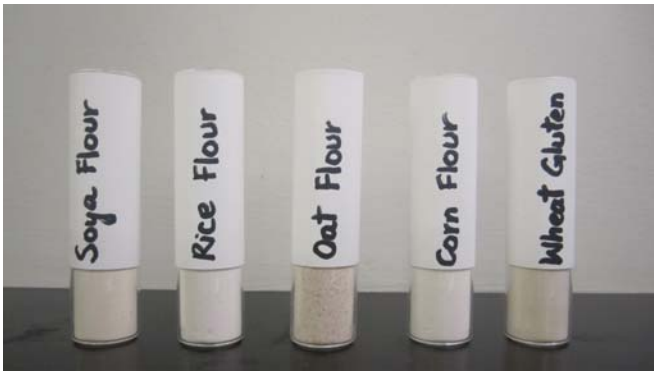

Figure 1. Various flour powders

- Various types of flour products such as biochoix, corn pasta (gluten-free), macaroni, penne quinoa (gluten-free) and penne with rice bran were obtained from different suppliers.

Instrumentation and method

All infrared spectra were recorded using the rugged portable Agilent Cary 630 ATR-FTIR analyzer (Figure 2).

The following procedure was used for all data acquisition:

1. A small amount of flour (without any sample preparation or weighing) was placed on the ATR diamond surface.

Figure 2. Agilent Cary 630 ATR-FTIR analyzer

2. The samples were pressed against the diamond crystal using the attached pressure clamp. A slip clutch on the clamp prevents overtightening.

3. Spectra were recorded by adding 64 spectra with a resolution of 4 cm^{-1} (measurement time ~30 seconds).

Results and discussion

Figure 3 shows several overlaid infrared spectra for selected flour samples. The infrared bands characteristic of major flour components such as proteins, carbohydrates, lipids and moisture content can be clearly discerned in the spectra. The intuitive Agilent MicroLab FTIR software provides the means by which sample spectra can be compared to spectra of flour standards already stored in a spectral database of flours. A spectral database (library) can be quickly and easily created on-the-fly in MicroLab PC. A new sample can then be identified immediately after recording its infrared spectrum (Figure 4).

The Agilent Cary 630 ATR-FTIR analyzer can be configured to report a pass/fail or percentage (%) spectral similarity with regard to a reference sample stored in the spectral database. Moreover, the spectral similarity among the infrared spectra of incoming new materials and previously recorded samples is particularly valuable in tracking batch-to-batch or lot-to-lot variability from the same or different vendors.

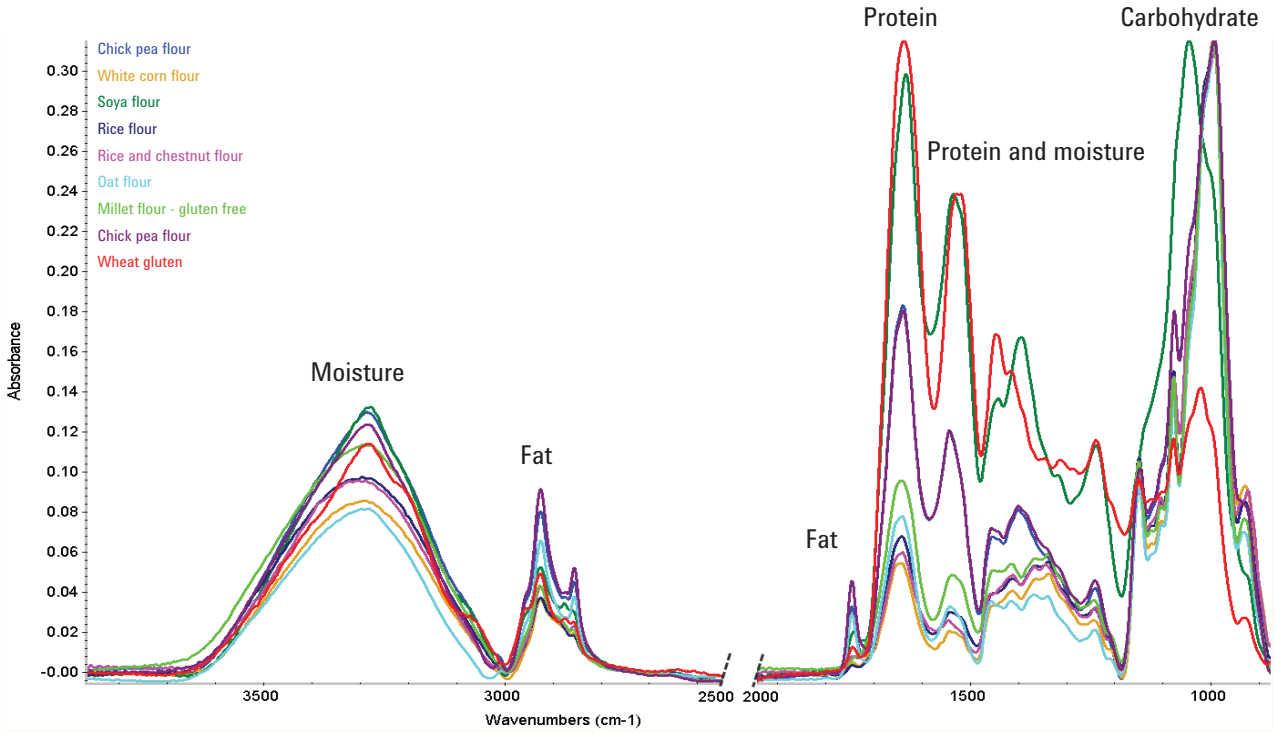

Figure 3. Overlapped infrared spectra of selected flour powders recorded on the Cary 630 ATR-FTIR analyzer

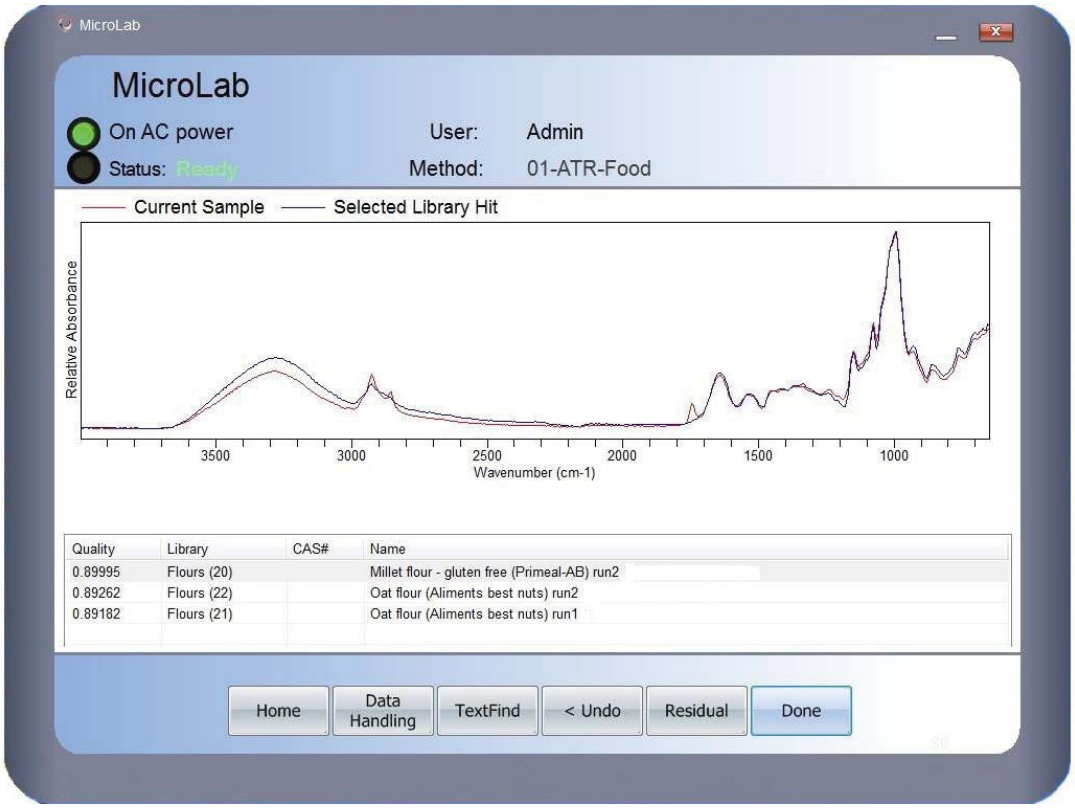

Figure 4. Agilent MicroLab FTIR software correct identification of an unknown flour sample

Conclusion

The Agilent Cary 630 ATR-FTIR analyzer is an FTIR spectrometer with a built-in rugged diamond surface. It rapidly produces high quality spectra and it provides a rapid and robust method to differentiate between different flours. Moreover, the Cary 630 ATR-FTIR is quite portable and can be equally employed at the receiving dock, in the storage facility or in the process line to ensure the highest possible product consistency.

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Agilent Technologies

QA/QC of sugars using the Agilent Cary 630 ATR-FTIR analyzer

Application note

Food testing and agriculture

Zubair Farooq and Ashraf A. Ismail

McGill University
McGill IR Group
Department of Food Science and Agricultural Chemistry
Montreal, Quebec
Canada

Introduction

Various manufacturers especially in the chemical, pharmaceutical and food industries employ sugars in their products. Sugars can be extracted from plant or microbial sources or they can be chemically synthesized. The majority of sugars are purchased as crystalline white powders and the most common examples are glucose, sucrose, lactose, fructose, maltose and xylitol. Protein-based sweeteners such as thaumatin, curculin and monellin are also used. Substituting artificial sweeteners such as aspartame, saccharin and sucralose can significantly reduce or virtually eliminate the calories associated with sugar content in food and beverages.

Due to the similarity in their color, odor, texture and general appearance (Figure 1) it is very difficult for processors to authenticate the sugar identity prior to its addition to a formulation.

Currently, most pharmaceutical and food manufacturers rely on chromatographic techniques, which are tedious, time-consuming and require the use of solvents, to distinguish between the sugars. Occasionally, for quality assurance/quality control (QA/QC) purposes it is necessary to use internal or external chemical laboratory support for testing. As in all food commodity or food-grade pharmaceutical excipients, the manufacturer usually requires an advance sample from the supplier to verify that it meets their requirements. The sample is analyzed to compare it with other lots previously received. Then, when the bulk shipment arrives it must be tested to ensure that it has the same chemical and crystalline composition as the advance sample and that it is homogenous in composition. This can be accomplished by acquiring multiple samples from different containers. Sometimes, material from the regular supplier is not available and a new vendor must be found and the new sugar chemical composition must be compared to that supplied by the previous vendor.

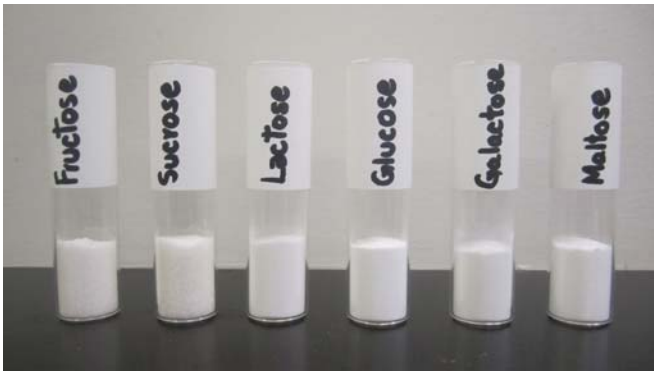

Figure 1. Various sugars

Manufacturers who use sugars in their products need a ready-to-deliver, robust technology, free from system calibrations, that can test and accept or reject the sugar powder on the spot, ideally immediately on receiving the sample.

Experimental

Small amounts of sugar samples were placed without weighing onto the sampling surface of the Agilent Cary 630 ATR-FTIR analyzer (Figure 2). The samples were pressed against the diamond crystal using the attached pressure clamp. A slip clutch on the clamp prevents overtightening.

Spectra were acquired in about 30 seconds by co-adding 64 spectra with a resolution of 4 cm^{-1}. Each resulting spectrum was displayed on the computer screen along with its closest spectral analogue chosen from a spectral library database of sugar standards acquired using the Cary 630 ATR-FTIR analyzer and its MicroLab FTIR software.

Figure 2. Agilent Cary 630 ATR-FTIR analyzer

Results and discussion

All carbohydrate-based sugars possess strong and characteristic infrared absorptions between 1200 and 600 cm^{-1} attributed to C-O-H and C-O-C bonds (Figure 3, top). Protein-based sweeteners like thaumatin show unique absorption bands between 1700 and 1500 cm^{-1} (Figure 3, bottom), which can be attributed to the amide I and amide II absorption bands belonging to the proteins. Artificial sweeteners also possess unique absorptions depending on their chemical structure. Accordingly, the spectrum of a sugar can be easily assigned to a particular sugar class (carbohydrate, protein-based or artificial sweetener) and in most cases assigned with a high degree of accuracy to a specific sugar. Hence, when a new or unknown sweetener is received, its spectrum can be compared to those stored in the database of previously recorded sweeteners, and its identity can be correctly established by the MicroLab FTIR software (Figure 4).

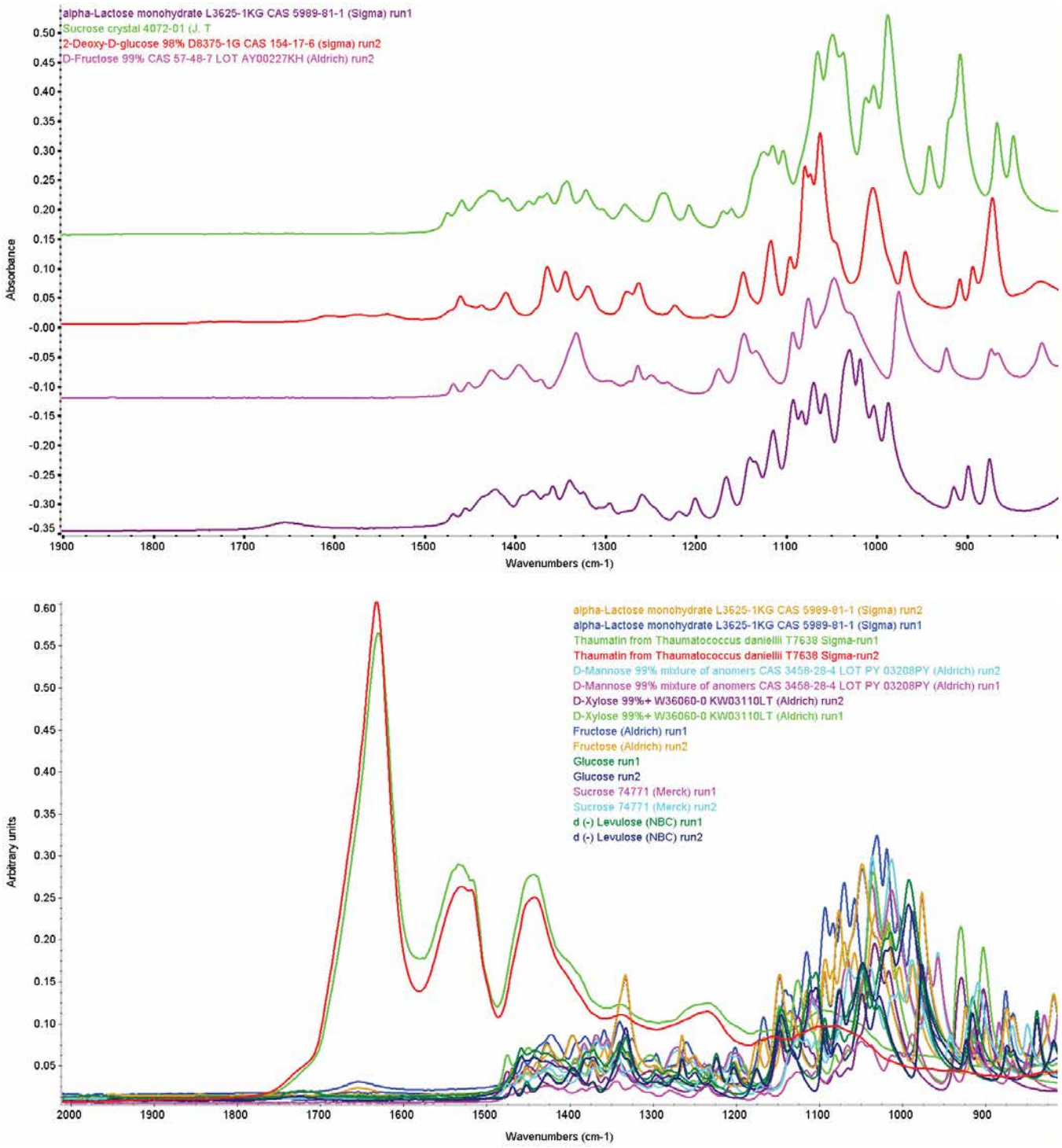

Figure 3. Infrared sugar spectra recorded on the Cary 630 ATR-FTIR analyzer. Spectra in the upper image have been offset. The strong infrared absorption band in the lower image located between 1700 and 1600 cm^{-1} is attributed to the amide I band of protein sweeteners.

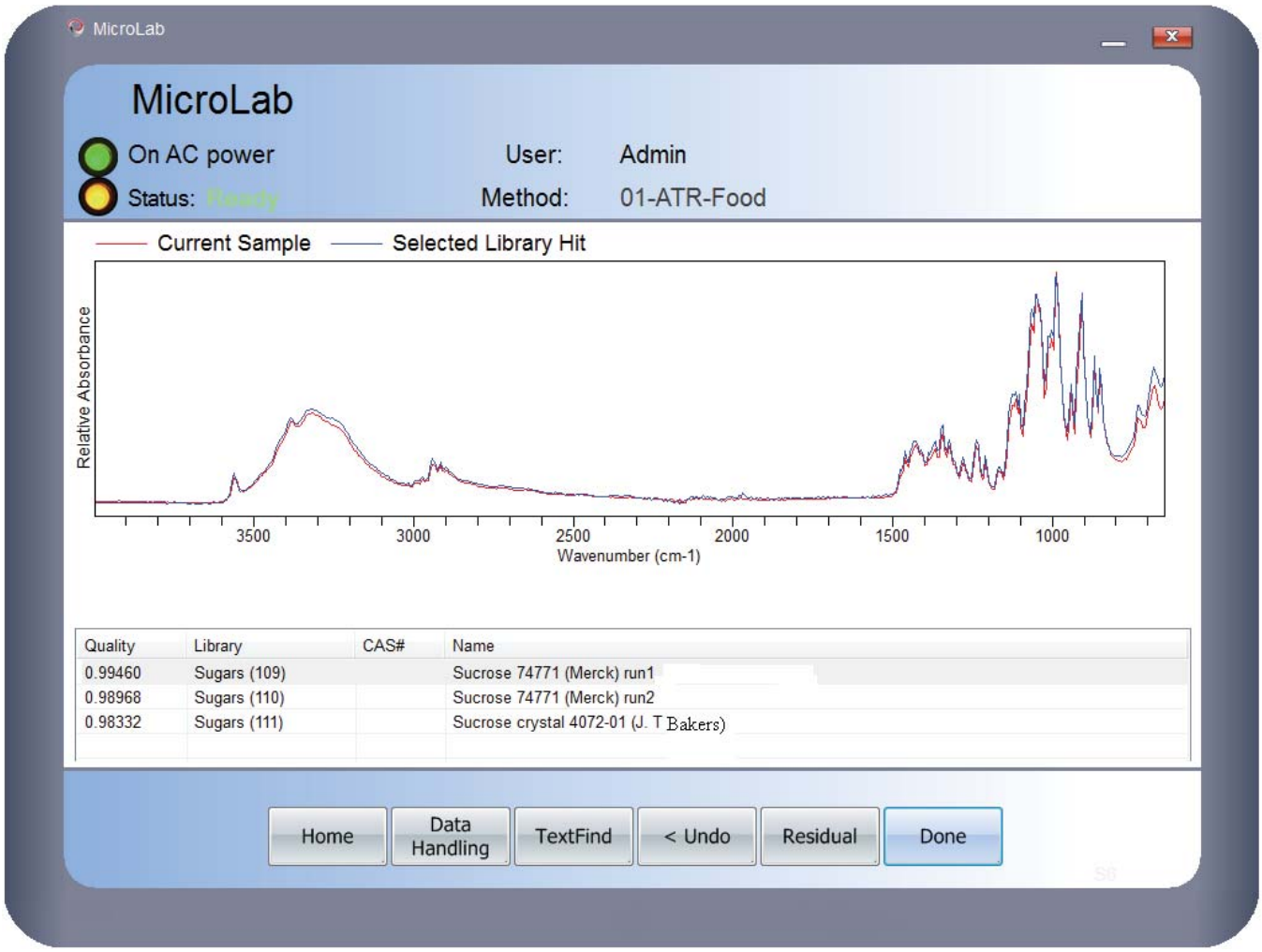

Figure 4. Correct identification of an unknown sugar sample by the MicroLab FTIR software

Conclusion

The Agilent Cary 630 ATR-FTIR is a robust, light-weight and compact (12 in long, 6 in wide, 9 in high and ~7 lb) analyzer that rapidly provides high quality spectra. It comes with very intuitive MicroLab FTIR analysis software for authenticating sugar samples by comparison to known spectra of reference standards. This makes the Cary 630 ATR-FTIR analyzer ideal for use at the receiving dock, on the production floor or it can be taken to the ingredient supplier depot for on-site verification.

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Agilent Technologies

QA/QC of tea using the Agilent Cary 630 ATR-FTIR analyzer

Application note

Food testing and agriculture

Zubair Farooq and Ashraf A. Ismail

McGill University
McGill IR Group
Department of Food Science and Agricultural Chemistry
Montreal, Quebec
Canada

Introduction

Tea is the most widely consumed beverage after water. Teas are generally divided into categories based on processing techniques. The most commonly applied processes are oxidation and fermentation. There are many different tea varieties (Figure 1) including white, yellow, green, oolong, black and post-fermented. Some teas can be modified further by the addition of additives or by blending different varieties to improve taste. Almost all teas sold in bags and other types of teas (leaves) in the world are blends.

Tea leaves contain more than 700 compounds including, flavanoids, amino acids, vitamins (C, E and K), caffeine, polysaccharides, flavanols, tannin, polyphenols and catechins. Catechins can make up to 30% of the dry weight of teas and their concentration is greatest in white and green teas, while black teas contain substantially fewer catechins.

The levels of antioxidants in green and black teas do not greatly differ. In addition, teas contain neurologically-active compounds such as theanine, theophylline, theobromine and caffeine. The complexity of the chemical composition of teas makes it very difficult to establish an authentication process and quality control/quality assurance (QA/QC) procedures. Indeed, most companies will focus simply on a two-component analysis consisting of caffeine and the total polyphenol content. A complete component analysis would be highly desirable if it could be performed quickly and with minimal analysis cost per sample. Furthermore, companies that blend the teas would also benefit substantially if the authentication and quality verification of the arriving teas could be established prior to blending, to ensure the consistent production of high-quality products.

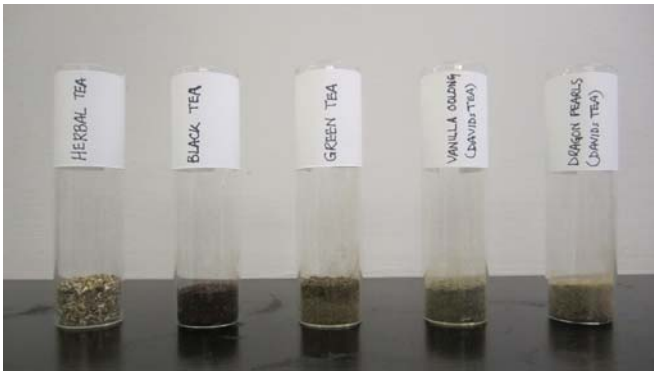

Figure 1. Various teas

Among available techniques, Fourier transform infrared (FTIR) spectroscopy used in combination with a diamond crystal attenuated total reflectance (ATR) accessory can provide important information about all the organic compounds present in teas, and this spectral information can be utilized in a number of ways to aid tea manufacturers in optimizing their products and to ensure product consistency. The Agilent Cary 630 ATR-FTIR analyzer can reduce operational costs, is lightweight (~7 lb or ~3 kg) and robust, and can thus be easily carried and used as a portable analyzer. The Agilent MicroLab FTIR software is easy-to-use and requires minimal training so that different tea varieties can be differentiated within minutes.

Experimental

1. Several different commercial tea brands were purchased from grocery stores and were ground to produce uniformly-sized powders.

2. A small amount of each ground-tea powder was placed on the diamond ATR crystal of the Cary 630 ATR-FTIR analyzer (Figure 2).

3. The samples were pressed against the diamond crystal using the attached pressure clamp. A slip clutch on the clamp prevents overtightening.

Figure 2. Agilent Cary 630 ATR-FTIR analyzer

4. FTIR spectra were acquired (64 scans at 4 cm^{-1} resolution) in less than 30 seconds.

5. The recorded spectra were automatically stored in a spectral database for comparison to previously pre-recorded reference spectra already in the spectral database or for the determination of a percentage (%) similarity comparison by the MicroLab FTIR software.

Results and discussion

All the ground-tea samples were scanned using the Cary 630 ATR-FTIR analyzer. The acquired spectra (Figure 3) were automatically stored in a spectral database using the MicroLab FTIR software. The spectral information between 3800 and 2600 cm^{-1} and 2000 and 600 cm^{-1} provides the means for differentiating between the different tea varieties. These spectral regions contain

absorption bands for all the major and minor organic components found in teas and therefore, all the spectral information within these regions plays an important role in distinguishing amongst teas with varying chemical compositions.

Each sample was run in duplicate sets (on different days) wherein one set was stored in a spectral database and the second set was used for validation purposes. In all cases, the duplicate samples were correctly identified and matched by the MicroLab FTIR software. Therefore, when a new sample is acquired, it can easily be compared to the previously recorded spectra, and its identity established within seconds of spectral acquisition by the MicroLab FTIR software (Figure 4). The results can be expressed in the form of a % similarity or pass/fail based on an operator's pre-established % similarity limits.

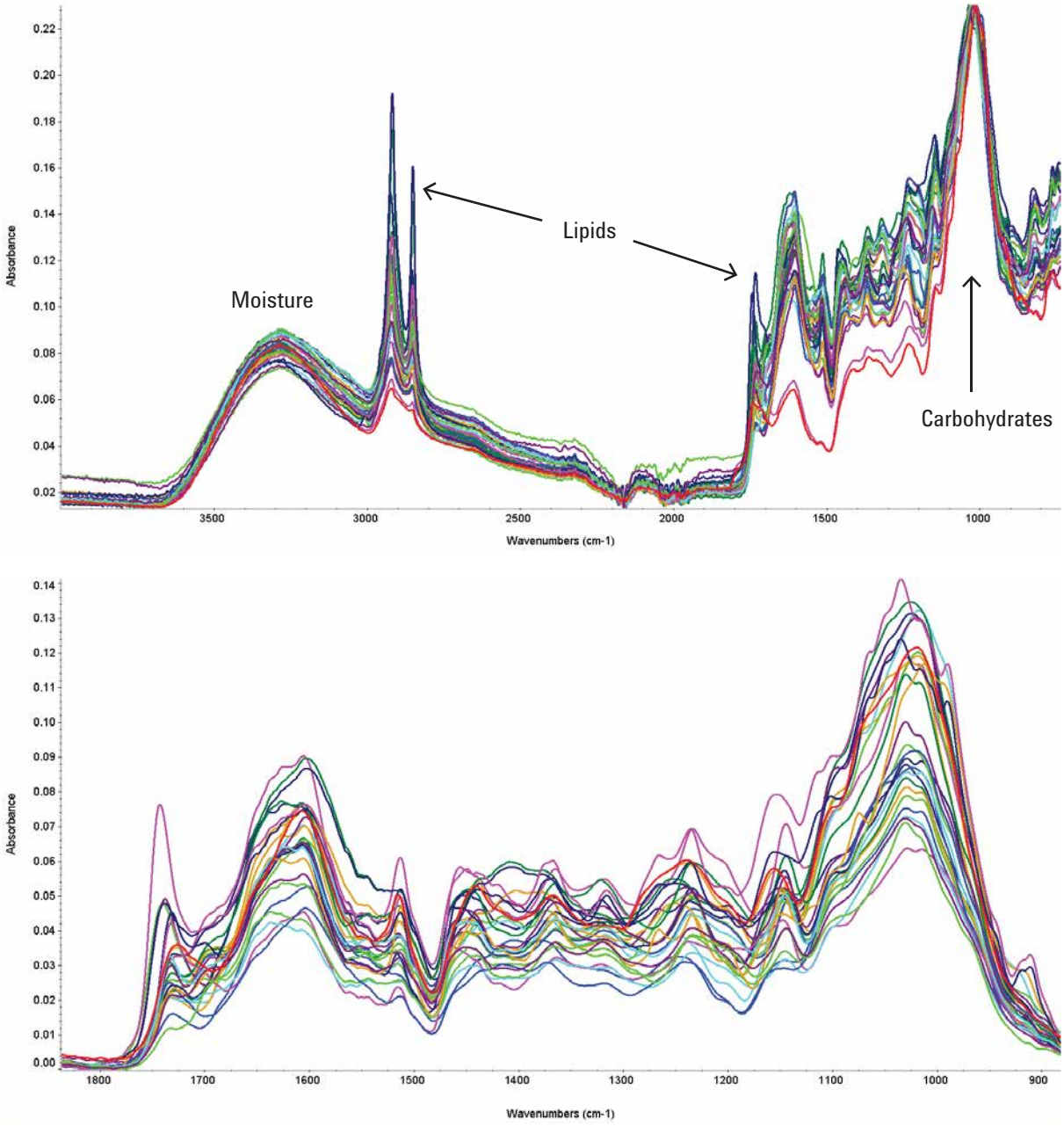

Figure 3. Agilent Cary 630 ATR-FTIR spectra of selected teas

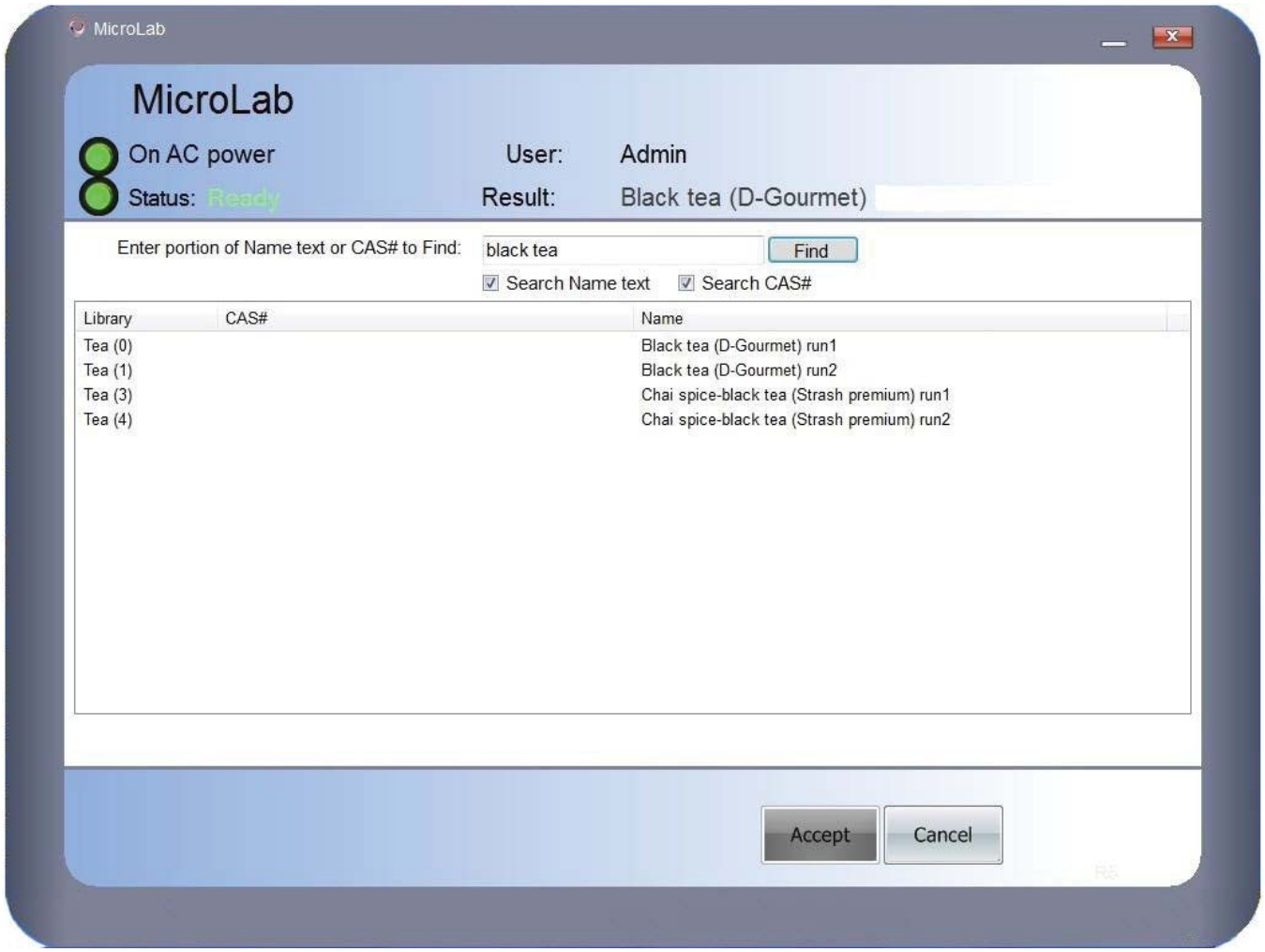

Figure 4. Example of the Agilent Cary MicroLab FTIR software for the correct identification of a new black tea sample

Conclusion

The Agilent Cary 630 ATR-FTIR analyzer can meet the challenge of providing a molecular fingerprint for all the organic components in teas. It is an extremely rapid, portable method that can provide analytical results within seconds without the use of solvents or any prior sample preparation. It can be employed at the receiving dock, in the production line, in the QC lab or it can be easily moved from one location to another. The resourceful MicroLab FTIR software allows the operator to carry out the analysis with minimal training. Since the operator receives real-time results he or she can either accept or reject a new tea shipment, verify the quality of materials held in storage, or ensure product consistency after blending.

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Agilent Technologies

At-Site Screening and Measurement of Adulterant Levels in Bovine Milk by Mid FTIR Spectroscopy

Application note

Food, QA/QC

Authors

Alan Rein, Ph.D.
Agilent Technologies, Inc.

Professor Luis Rodriguez-Saona
Food Science and Technology
Department of Ohio State University

Introduction

Milk is a common target for adulteration, which is of significant concern to both producers and consumers. Some common milk adulterants include water, whey, sodium hydroxide, urea, melamine and other potentially harmful substances. The purpose of adulterating milk is to artificially increase the volume and/or mask inferior quality product for economic gain.

For this reason, there is significant interest in rapid, easy to use analytical methods that can detect if milk is adulterated and then measure the levels of the adulterants in milk. In a recent publication [1], researchers measured adulterants in milk in the laboratory using the Agilent Cary 630 FTIR spectrometer and showed that the mid FTIR system is superior to NIR spectroscopy for these determinations.

With the recent availability of easy-to-use, dedicated FTIR analyzers, screening milk for adulteration and then measuring the specific contaminant levels is easier and faster than with traditional analytical methods. These FTIR analyzers are designed for use in at-site production locations by less experienced personnel and thus offer the dairy industry a means to improve productivity.

Compact, at-site FTIR systems for methods development and methods deployment in the dairy industry

Agilent now offers a family of ultra-compact FTIR spectrometers and analyzers (Figure 1), which all utilize the same optics, software and sampling technology [2]. If multi-purpose methods development and QA/QC is of primary interest, the Cary 630 FTIR spectrometer is ideal. For deployment of specific FTIR based solutions, the Agilent 5500 FTIR analyzer is an excellent platform for routine at-site analysis. Both of these systems use diamond ATR sampling technology for the analysis of solids and films; Agilent's exclusive DialPath transmission sampling technology is used for the analysis of liquid milk samples.

Figure 1. Agilent Cary 630 FTIR spectrometer for routine QA/QC and methods development and the Agilent 5500 FTIR analyzer for deployment of methods in at-site production labs.

This application note will describe two distinct methods for measuring adulteration in milk:

- A screening method to detect that an adulteration has taken place, using an Agilent FTIR analyzer equipped with DialPath sampling technology
- Identification and measurement of specific adulterants, using an Agilent FTIR analyzer equipped with diamond ATR sampling technology

In the screening method, the milk is measured directly with no sample preparation, leading to an extremely fast analysis. Using this technique on the dedicated Agilent 5500 or 4500 FTIR spectrometer allows for efficient screening at the point of delivery.

To get accurate identification and measurement of the adulterant, a second method is employed that uses simple sample preparation steps. This latter technique provides a fast alternative to classical analytical methods of determining milk contamination levels.

Experimental

Instrumentation

Table 1. Spectrometer parameters used for both screening and specific identification methods

Parameter	Settings
Screening	DialPath technology 30 micron pathlength
Measurement	ATR technology single reflection diamond ATR
FTIR spectra	64 co-added interferograms
Resolution	4 cm^{-1}
Measurement time per sample	Approx. 30 seconds

Materials and reagents

Preparation of milk standards for analysis

Commercial bovine milk samples were mixed with varying amounts of tap water, whey, synthetic milk, synthetic urine, urea and hydrogen peroxide. These materials were chosen because they are reported as common adulterants in certain countries for either increasing volume, adding nitrogen (to provide better results from Kjeldahl protein assays) or to sanitize the milk (hydrogen peroxide).

The overall dilution of the milk by adulterants was from 3 to 50% v/v. The concentrations of specific adulterants spiked into milk samples was: 1.87 – 30 g/L for whey; 0.78 – 12.5 g/L of urea and synthetic urine; 0.05 – 0.8 g/L of urea for synthetic milk and 0.009 – 0.15 g/L for hydrogen peroxide. Six lots of milk were adulterated with five levels of contaminants resulting in 30 adulterated samples per lot.

For adulerant screening, two drops each of the spiked liquid samples were measured with the DialPath sampling technology (Table 1).

For measuring each adulterant individually, the spiked samples were mixed in equal volumes with chloroform to extract the fat matrix interference. The water soluble supernatant was then applied to the diamond ATR sensor and vacuum dried to form a film (Table 1).

Results and discussion

Screening

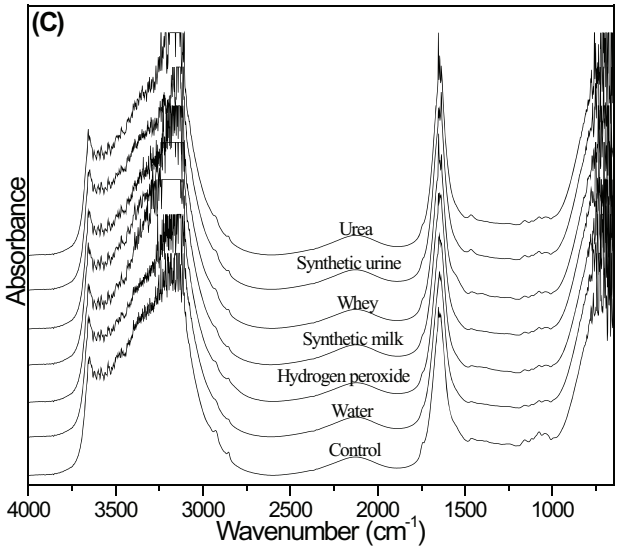

Figure 2. MIR spectra of diluted milk are dominated by contribution from water. Using a 30 micron pathlength allows MIR fingerprint region spectral information to be used for analysis.

The spectra of the samples are dominated by the strong absorbance from water. Whereas the O-H stretch centered at 3300 cm^{-1} is fully absorbing of MIR light, the information rich fingerprint region from 2000 to 800 cm^{-1} contains accessible information.

A partial least square model was developed based on overall dilution levels and good correlation was found between the infrared estimated concentrations and the level of dilution that resulted from the spiking process (Table 2).

Cleaning the DialPath sampling technology consists of simply wiping the windows in preparation for the next milk sample. This screening method takes less than two minutes from introduction of sample to clean-up, providing a highly efficient means of screening milk for dilution as a result of one or more contaminants. Agilent's Microlab software uses color coded alerts to advise the operator if the milk sample is diluted.

Analysis of specific contaminants in milk

The dried milk film showed major differences between the control sample and the spiked samples. For example, milk spiked with whey showed two strong absorbances associated with the protein Amide I and Amide II bands at 1635 cm^{-1} and 1530 cm^{-1}, respectively. Samples adulterated with urea, synthetic milk and urine exhibited strong bands at 1615 cm^{-1} and 1454 cm^{-1} arising from C=O and NH_4^+ absorbances.

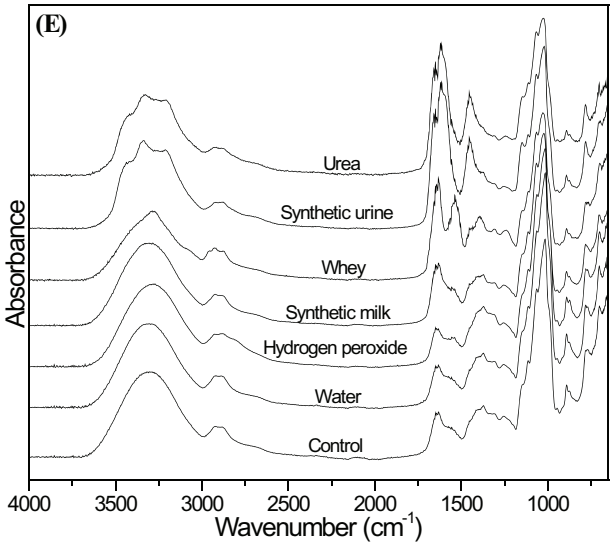

Figure 3. Spectra of dried milk film recorded with FTIR spectrometer using single reflection ATR

Partial least square regression analysis of the dried films (Table 3) demonstrates good correlation between the infrared estimated concentrations and the spike adulterant levels. Prediction ability of the models showed SEP values of 1.18 g/L (whey), 0.009 g/L (hydrogen peroxide), 0.028 g/L (synthetic milk), 0.412 g/L (synthetic urine) and 0.232 g/L (urea). These excellent results, as evidenced by low SEP, result from the ability to differentiate specific MIR absorbance bands for each of the adulterants used in the study.

Table 2. Results from PLS model correlating level of dilution with mid-infrared fingerprint region spectra

	Number of milk lots	Number of samples	Factors	SEC	SECV	SEP	R^2cal	R^2val
Agilent FTIR (1300 – 950 cm^{-1})								
All adulterants	4	372	4	0.74	0.76	0.83	0.98	0.98

Table 3. PLS results from spectra of dried milk measured by Agilent FTIR analyzer equipped with single reflection diamond ATR

	Number of milk lots	Number of samples	Factors	SEC	SECV	SEP	R^2cal	R^2val
Whey	5	90	5	1.03	1.16	1.18	0.98	0.98
Hydrogen peroxide	5	90	4	0.008	0.009	0.009	0.96	0.94
Synthetic milk	5	90	5	0.023	0.027	0.028	0.98	0.98
Synthetic urine	5	90	4	0.333	0.364	0.412	0.98	0.98
Urea	5	90	5	0.175	0.210	0.232	0.98	0.98

SEC and SECV values in g/L

Conclusion

Agilent FTIR analyzers equipped with DialPath transmission sampling technology provide an easy-to-implement, rapid method for screening milk samples for tampering to as low as 3% v/v dilution. These systems equipped with single reflection diamond ATR sampling technology afford measurement of specific common adulterants in dry milk films. For laboratories located at dairy processing sites, this combination of technology and methodology offers a time and cost saving alternative to traditional methods of milk analysis.

References

1. P. M. Santos, E. R. Pereira-Filho, L. E. Rodriguez-Saona, "Application of handheld and portable infrared spectrometers in bovine milk analysis", Journal of Agricultural and Food Chemistry, February 13, 2013, 61(6), 1205-1211.
2. "Agilent's FTIR family - lab results, anywhere you want" Agilent PN: 5991-1405EN

Agilent wishes to acknowledge Professor Luis Rodriguez-Saona's group at the Food Science and Technology Department of Ohio State University for methods, data and helpful discussion.

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Agilent Technologies

Pesticide Authentication by Portable FTIR Spectroscopy

Application note

Food

Authors

Shannon Richard
Alan Rein

Agilent Technologies,
Danbury CT, USA

Introduction

Counterfeit and illegally sold pesticides are a rapidly growing global problem, which affects food production, the health of farmers and consumers, and the overall environment including beneficial insects and animals. In Europe and the U.S, it is estimated that over a billion dollars in illegal pesticides are sold yearly. In some areas of the world, more than 25% of pesticides are counterfeit.

Counterfeit pesticides are generally classified[1] as: *fakes*, which contain no biologically active ingredients or are highly diluted or banned pesticides; *counterfeits*, which have packaging that is authentic in appearance but contain impure or incorrect chemicals or *illegal imports*, which are generic copies of legitimate products.

There is compelling need to eliminate the trade in illegal pesticides and there are ample rules and regulations, but not enough enforcement. With the increasing availability of mobile and portable analytical instrumentation, those agencies and personnel responsible for ensuring pesticide safety and usage have powerful new tools to address the problem.

In this application note, we demonstrate how a portable FTIR spectrometer can be used to rapidly analyze pesticides before distribution, before mixing, and/or before application to crops.

Advantages of Portable FTIR Analyzers for the Analysis of Pesticides

Mobile FTIR offers a number of advantages for personnel involved in ensuring the authenticity of pesticides:

- Mid infrared spectroscopy provides a detailed fingerprint of the chemical structure of the suspect pesticide.
    - Verifies identity of pesticides and identifies diluted, inert, banned or improperly identified agents.
    - Differentiates closely related pesticides based on spectral fingerprint.
- Analysis requires no sample preparation. Results on suspect samples are obtained in less than one minute using the on-board library of pesticides.
    - Large numbers of containers can be rapidly assessed in distribution, warehouse or other locations.
- At site analysis enables personnel to make real-time actionable decisions.
    - Off specification pesticides can be stopped before they are shipped or applied to a field.

Figure 1a. The Agilent 5500 FTIR analyzer equipped with diamond ATR sampling technology for use in at-site labs.

The Agilent 4500 FTIR and 5500 FTIR systems (Figures 1a and1b) are well suited to the analysis of pesticides:

- The 4500 FTIR system is fully portable and battery powered. This enables true at-site measurements of pesticides regardless of location.
- The 5500 FTIR system is a bench top system well suited to fixed, at-site labs or in mobile van based labs.
- Both systems utilize an ATR element as the sampling sensor; the diamond sensor is immune to chemical attack by corrosive substances. One drop of pesticide is required for analysis.
- The MicroLab software is intuitive and highly visual. Colored alerts alert the user to the presence of off-specification pesticide.
- The MicroLab software compares the spectrum of the suspect pesticide to spectra contained in an on-board library to instantly verify or disprove identity.

Material and Methods

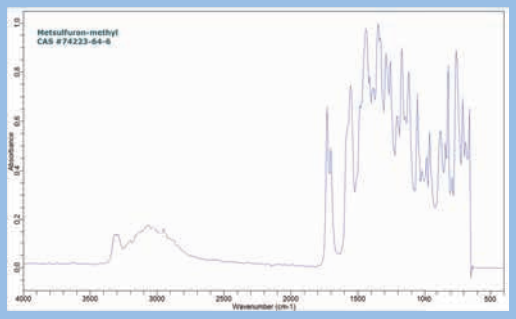
A series of pesticides were analyzed using the Agilent 4500a portable FTIR system outfitted with single reflection diamond ATR sensor. The pesticides that were analyzed included cis-chlordane, trans-chlordane, aldrin, lindane and campheclor. Approximately 0.5 µL of each pesticide was placed on the diamond ATR sensor. The recorded spectra consisted of 64 co-added interferograms measured at 4 cm^{-1} resolution. Spectra were searched against the a commercially available pesticides library from ST Japan, and the identity of the sample pesticide was determined in less than one minute

Figure 1b. The Agilent 4500 battery powered FTIR analyzer for similar measurements in out-of-lab applications.

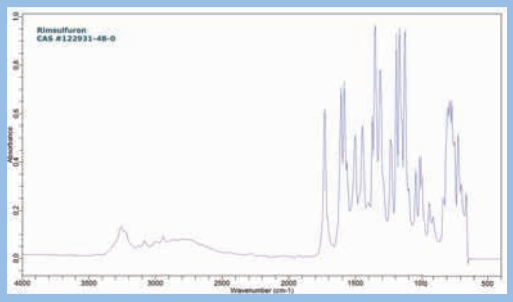
Confirming Pesticide Authenticity is Critical

In 2004, thousands of acres of crops in Spain, Italy and France were destroyed by the application of a counterfeit pesticide. The pesticide was offered at 20% discount and the packaging was identical to that of the expected product. In reality, the 20% discounted substance was metsulfuron-methyl and not the correct pesticide, rimsulfuron. The result was extensive destruction of produce and crops.

Metsulfuron-methyl and rimsulfuron are clearly distinguishable by their mid-infrared spectra.



Spectrum of Metasulfuron-methyl



Spectrum of Rimsulfuron

Portable FTIR aids in ensuring that correct pesticides are applied to fields and produce

Results and Discussions

The pesticides that were selected for this demonstration are banned or severely restricted, as they are classified as persistent organic pollutants. These are all chlorine containing compounds that are toxic to humans and animals, as well as to insects.

Cis and trans chlordane, which have similar structures, were readily identified by their respective spectra and comparison with the commercial pesticide library provided excellent matches (Figures 2, 3)

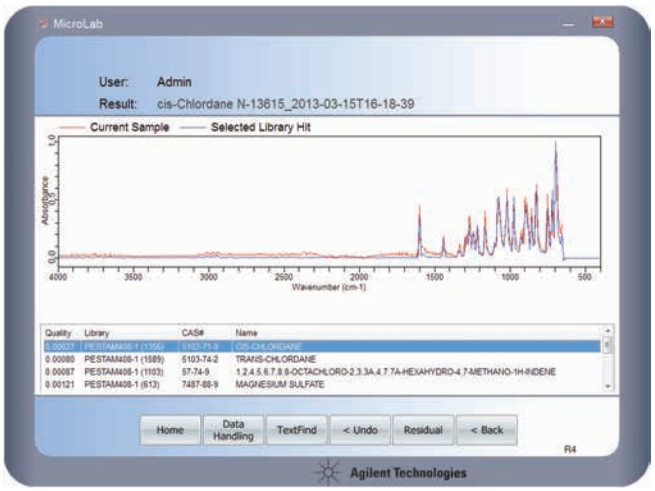


Figure 2. Identification of cis-Chlordane.

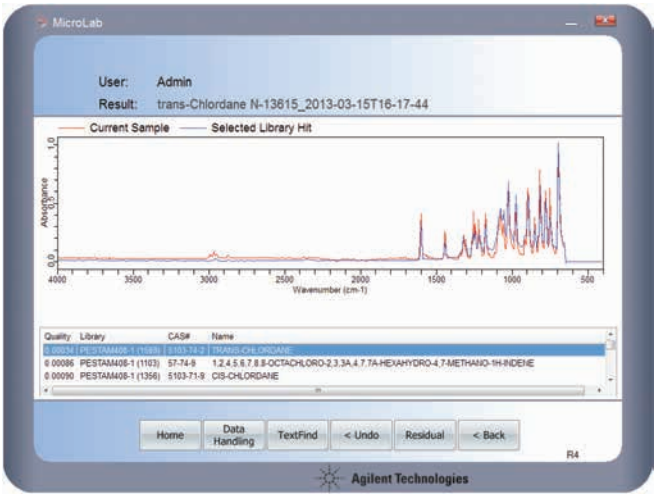


Figure 3. Identification of trans-Chlordane.

Campheclor, a highly toxic and carcinogenic substance that was banned by the Stockholm Convention, is a complex mixture of 200 different compounds formed by the chlorination of camphene. Despite the complexity of the formulation, the IR spectra was easily searched and the identity of the material was ascertained (Figure 4)

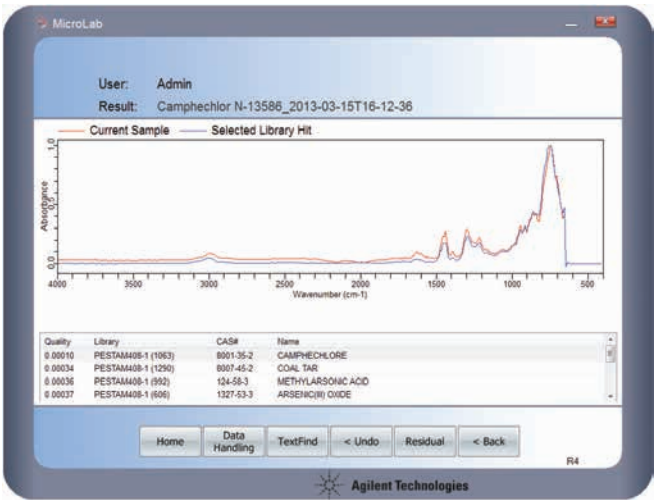


Figure 4. Identification of Campheclor.

Excellent matches were also obtained for lindane and aldrin, potent organochlorine insecticides also banned by the Stockholm Convention (Figures 5,6)

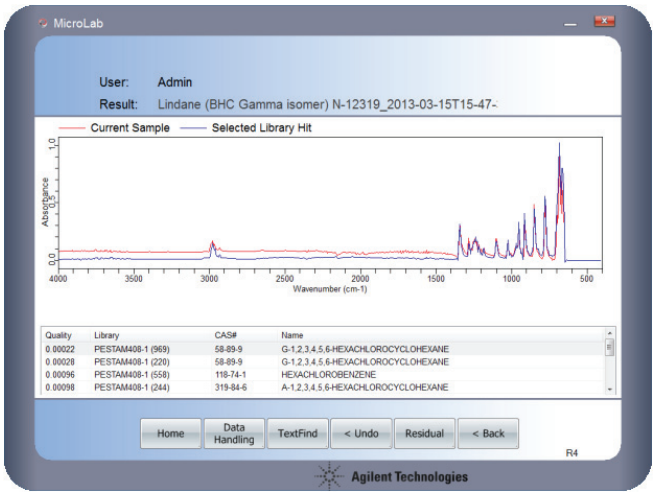


Figure 5. Identification of Lindane.

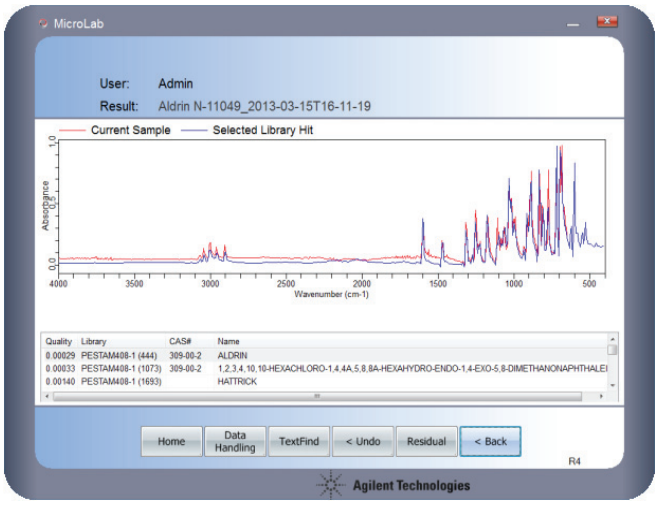


Figure 6. Identification of Aldrin.

Conclusion

FTIR spectroscopy can quickly and easily identify undiluted pesticides with a minimum of sample preparation. The combination of commercially available pesticide data bases combined with the performance and mobility of the Agilent 4500 and 5500 Series FTIR, enable rapid, on-site analysis of suspect product. Adulterated, contaminated or otherwise out-of-specification pesticides can now be analyzed in locations where they are manufactured, shipped, received, stored and sold. Once pesticides are diluted for application to crops, the FTIR analyzers are capable of identification if the active ingredient level is > 5%. For more dilute solutions, or for determining the level of residual pesticide in crops, other technologies are employed[2].

This technology offers port and border control officers the capability to spot check containers for content authenticity. This ensures that pesticide identity is verified prior to application in fields and farms and mitigates the risk of detrimental effects on health, produce, crops and the environment.

Suggested Configuration

0021-010 4500a FTIR Single Reflection ATR

G8046AA#117 ATR Pesticides Library from ST Japan

References

[1] Frederick Fishel, The Global Increase in Counterfeit Pesticides, Institute of Food and Agricultural Sciences, University of Florida (2009).

[2] Agilent Publication, Accelerated Analysis of On-Site Pesticide Detection in Vegetables by Agilent 5975T LTM GC/MSD and TSP, 5990-8067EN, June 8, 2011.

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Agilent Technologies

Measurement of Acrylamide in Potato Chips by Portable FTIR Analyzers

Application note

Food

Author

Alan Rein[1]
Professor Luis Rodriguez-Saona[2]

[1] Agilent Technologies,
Danbury CT, USA.

[2] Department of Food Science
and Technology
Ohio Sate University, OH, USA.

Introduction

Acrylamide is a compound that forms in french fries, potato chips, cereal, bread and coffee when they are fried, roasted or baked. The formation is dependent on cooking conditions, for example, high temperature over-cooking of fried chips results in very high levels of acrylamide. The substance, which forms from the reaction of sugar and amino acids in food, is reported to be a likely human carcinogen and formal regulation of acrylamide levels is under consideration worldwide. The compound is on California's Proposition 65 carcinogenic substances list, which requires a warning label on food products that contain elevated levels of a posted substance. Following legal action by the State of California, major potato chip manufacturers have agreed to reduce the level of acrylamide in potato chips to 275 ppb over the next several years. Acrylamide levels are of concern to European Union countries as well, and have been monitored in food for the past two years.

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The current methods for determining the level of acrylamide in foods use GC-MS and LC-MS/MS, which require significant sample preparation, specialized instrumentation and knowledgeable operators. Food producers often turn to third party laboratories for the measurement of acrylamide. Thus, there is a need for rapid, accurate and real-time measurement of acrylamide to allow monitoring and controlling of acrylamide formation during food processing.

In a recent article [1], it was shown that a portable FTIR gave equivalent and in some cases better measurement of acrylamide when compared to a traditional lab FTIR and NIR spectrometers. This result was accomplished using a method that requires far less sample preparation than the LC-MS/MS method. In this application note, we summarize the findings of that work and demonstrate that FTIR spectroscopy employing compact, easy to-use FTIR analyzers provides acrylamide level measurements that are equivalent to the referenced mass spectroscopy method with less sample preparation.

Figure 1a. The Agilent 5500 FTIR analyzer equipped with diamond ATR sampling technology for use in at-site labs measuring acrylamide.

Figure 1b. The Agilent 4500 battery powered FTIR analyzer for similar measurements in out-of-lab applications.

This combination of technology and methodology provides more rapid, at-site acrylamide measurements and provides information to help control the cooking process conditions. The FTIR procedure also provides an easier and faster quality control method for measuring acrylamide levels in finished foodstuffs.

Compact at-site FTIR systems to develop and deploy methods for detecting acrylamide

Portable FTIR analyzers have significant value to the food industry. With increasing globalization of food sources, quality and safety is of paramount importance to consumers, regulatory agencies and the companies involved in growing and processing food and food ingredients. The value of portable FTIR analyzers arises from the ability to:

- Identify and verify food ingredients when and where needed.
- Eliminate adulterated, counterfeit or out-of-spec ingredients.
- Detect, identify and measure contaminants in food and food ingredients.
- Get answers at collection, receiving and processing sites, as well as at farms or fields.
- Aid in keeping harmful or out-of-spec ingredients and foods “at the factory gate”.
- Allow actionable, on the spot decisions to proactively improve quality, safety and production efficiency.

Agilent offers a family of highly compact FTIR spectrometers and analyzers (Figure 1a and 1b), which all utilize the same optics, software and sampling technology [2]. If multi-purpose methods development and QA/QC is of primary interest, the Cary 630 FTIR spectrometer (not shown) is most suitable. For deployment of the FTIR based acrylamide method, the Agilent 5500 FTIR analyzer is recommended for at-site lab analysis. For analyses of acrylamide in non-lab environments, the Agilent 4500 battery powered FTIR analyzer is ideal. For the acrylamide FTIR method, these systems all employ diamond ATR sampling technology.

Experimental

Sixty-four different samples of potato chips were analyzed for acrylamide by infrared spectroscopy and by the referee mass spectroscopy method. To prepare the sample for infrared analysis, a hydraulic press compressed the potato chips into a "chip cake". This process forces excess oil out of the potato chips and reduces the strong infrared absorbance bands arising from the vegetable oil component. For each 10 g sample of potato chips, three chip cakes were prepared and the spectra were recorded. The chip cake was ground into a powder that was placed on a single reflection diamond ATR equipped portable Agilent FTIR analyzer. The resultant spectra consist of 64 co-added interferograms recorded at 4 cm^{-1} resolution in the 4000–700 cm^{-1} wavelength region.

Analyzer control and data acquisition was executed using Agilent Technologies MicroLab software and collected spectra were imported into Pirouette software (Infometrix Inc., Woodville WA) for multivariate analysis. The spectra were normalized and second derivative transformed via a Savitsky-Golay second order polynomial filter with a 25-point window prior to the partial least squares regression analysis (Figure 2).

Quantitative results were obtained by correlating the IR spectra with the acrylamide concentrations, as determined by the reference LC-MS/MS method. For independent validation studies of regular potato chips (not seasoned or sweet potato chips), 75% of the sample set was used for generating the calibration and the remainder served as the independent validation set.

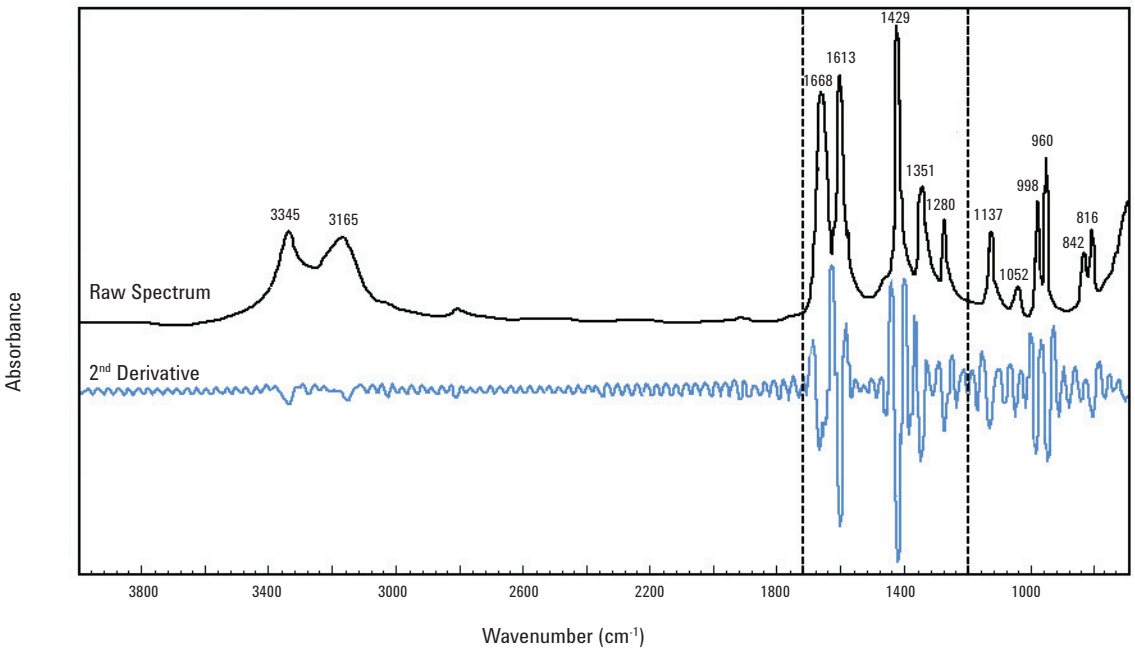

Figure 2. MIR spectrum and second derivative plot of acrylamide.

Results and Discussion

The acrylamide levels in potato chips, as determined by the reference LC-MS/MS method varied from 169 to 2453 µg/kg (169 to 2453 ppb). LOD and LOQ concentrations for the mass spec method were calculated to be 18 and 55 micrograms/liter.

Loading plots of the first two factors in the PLS correlation showed that the highest relevant variation in the calibration set for acrylamide content was in the range of 1201–1699 cm^{-1}.

The strong 1730 cm^{-1} absorbance band in the spectrum of potato chip cake (Figure 3) arises from fat, and is excluded in the PLSR model.

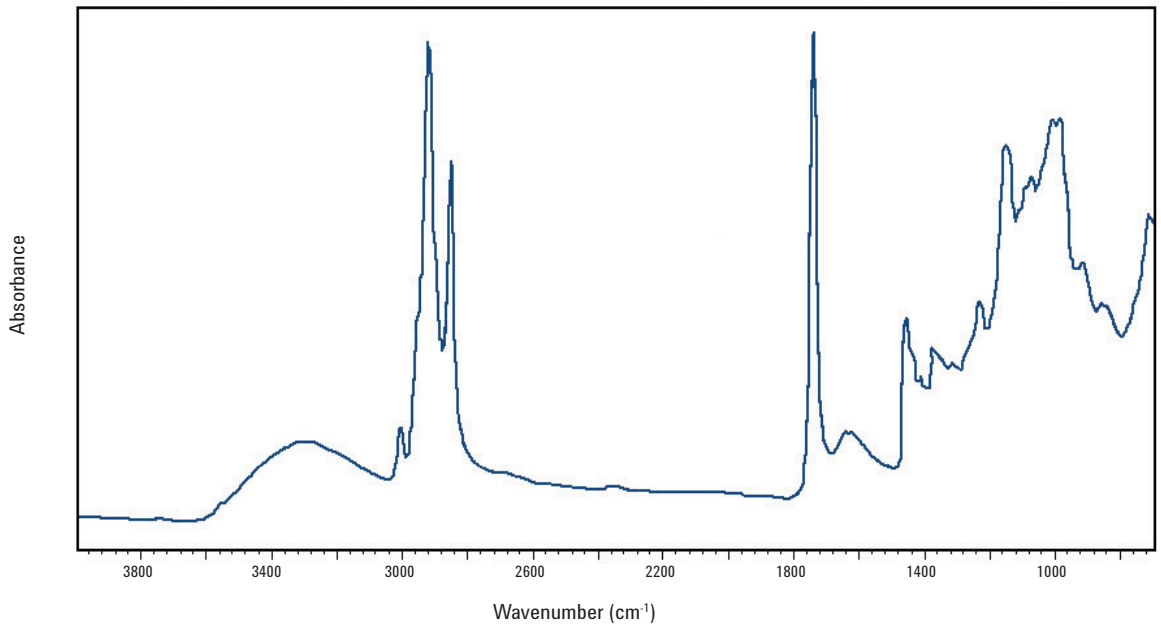

Figure 3. Spectrum of regular potato chip cake as measured by portable FTIR analyzer equipped with single reflection diamond ATR sample technology.

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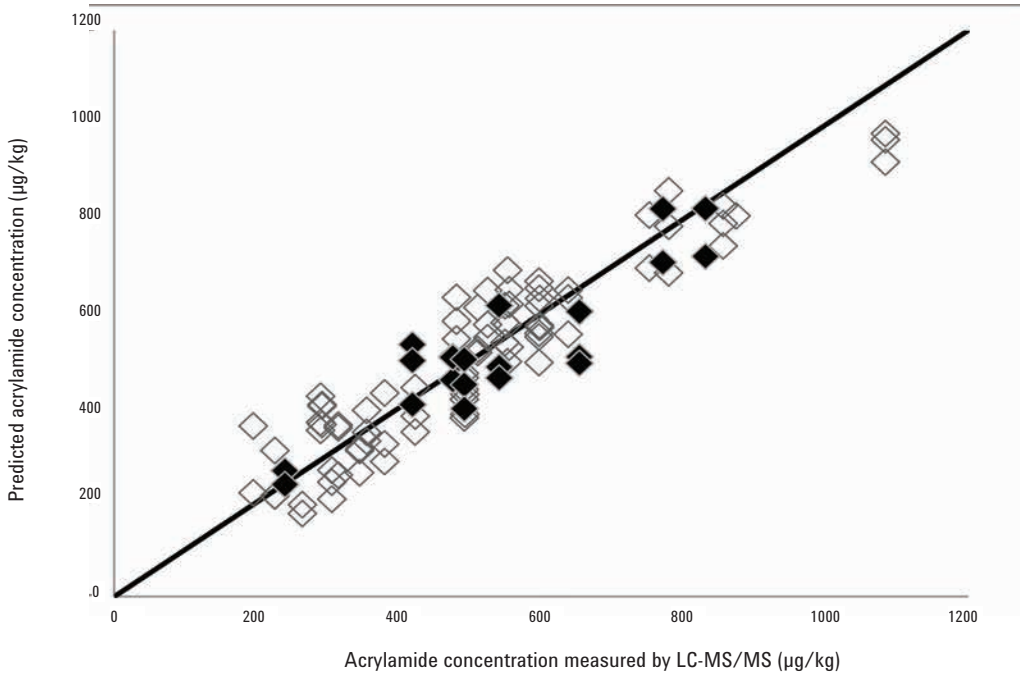

Figure 4. Correlation between infrared estimated levels and reference acrylamide values. The white squares represent samples in calibration groups; black squares represent samples used in validation groups.

Table 1. Calibration, cross-validation and prediction results of PLSR models developed by using mid-infrared instruments.

Sensor	Potato Chip Type		Factors	SE (µg/L)	r
Portable Cary 630 MIR	[a]Regular	Calibration	7	65	0.95
		Cross Val.	7	74	0.93
		Prediction*	7	75	0.90
	[b]Seasoned	Calibration	7	59	0.96
		Cross Val.	7	75	0.92
	[c]Sweet	Calibration	7	74	0.99
		Cross Val.	7	98	0.98

[a] "Regular" refers to potato chips containing only potatoes, vegetable oils and salt.
[b] "Seasoned" refers to potato chips containing additional ingredients.
[c] "Sweet" refers to sweet-potato chips.
* independent variable predictions made on regular potato chips only

The portable FTIR analyzer system equipped with single reflection diamond ATR sampling interface performed exceptionally well (Figure 4 and Table 1) with a SEP value for regular potato chips of 75 µg/kg (regular chips contain only potatoes, vegetable oils and salt).

Seasoned and sweet potato chip groups had SECV of 75 and 98 µg/kg, respectively. These ppb level measurements are consistent with current "acceptable" amounts of acrylamide in potato chips.

Conclusion

Agilent portable FTIR analyzers provide quantitative measurement of acrylamide levels in potato chips that is equivalent to, or better than, results obtained from traditional bench lab MIR or NIR spectrometers. The measurement of acrylamide in potato chips by FTIR provides a simpler analysis method for this potentially hazardous by-product than the current mass spectroscopy approach. The FTIR method eliminates consumables such as solvents, cartridges and columns and is far more efficient than the mass spectroscopy method. The level of user expertise necessary to obtain reliable results is not as critical with the FTIR method and, most importantly, it can be deployed wherever acrylamide needs to be measured, whether in a lab, a potato chip production facility, or for spot check measurements to determine levels in consumer product.

Acknowledgement

The data and results presented in this application note were provided by Professor Luis Rodriguez-Saona's research group at the Food Science and Technology Department of Ohio State University.

References

[1] Ayvaz, H. and Rodriguez-Saona, L., Application of Handheld and Portable Spectrometers for Screening Acrylamide Content in Commercial Potato Chips, submitted for publication to the Journal of Agricultural and Food Chemistry (2013).

[2] Agilent's FTIR family–lab results, anywhere you want" Agilent Publication 5991-1405EN, October 26, 2012.

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Agilent Technologies

Determination of phosphorus concentration in hydroponics solution

Application Note

Author

Sharon Lim

Agilent Technologies, Inc.
Mulgrave, Victoria 3170,
Australia

Introduction

Phosphates exist in many forms in water. They can occur as orthophosphates, condensed phosphates such as pyro, meta and polyphosphates or as organically bound phosphates.[1] The major pollution sources of phosphates in natural waters are detergents used in industry and fertilizers from agricultural farming.

The use of fertilizers is increasing. Phosphates in fertilizer and farm runoffs have to be monitored in order to prevent the possibility of polluting the surrounding environment. The excessive enrichment of natural waters with plant nutrients, known as eutrophication, can result in overabundant growth of undesirable algae and aquatic plants, affecting water quality.[2,3]

Solution spectrophotometry still remains an essential and reliable analytical method for routine water analysis, particularly for the determination of nutrient elements such as nitrogen and phosphorus.

Spectrophotometric methods often require the initial conversion of phosphates to soluble orthophosphates which can then be determined colorimetrically. Orthophosphates can be analyzed quantitatively by three methods:

1. Vanadomolybdophosphoric acid method
2. Stannous chloride method
3. Ascorbic acid method

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Methods 1 and 2 were used in this study to quantitate and confirm an unknown phosphorus concentration in a hydroponics solution. These methods are suitable for determining the level of phosphates such as KH_2PO_4 and $NH_4H_2PO_4$ added into fertilizers and hydroponics as sources of phosphorus. It is also important that the phosphate level conforms to plant requirements. If excess phosphates are used, this could lead to the death of plants as well as excess phosphate in runoffs.

Equipment

Instrument: Cary 1

Concentration Application software

Methodology

Digestion

Pretreatment of the hydroponics sample

The hydroponics sample was digested first, using the potassium persulfate method as described in reference 1. Acid digestion is necessary if total phosphorus is to be determined as phosphorus may be present in combination with organic matter.

Apparatus

1. Hot plate
2. Weighing boats

Reagents

1. Phenolpthalein indicator aqueous solution
2. Sulfuric acid solution (300 mL conc H_2SO_4 in 1 L H_2O)
3. Potassium persulfate $K_2S_2O_8$
4. Sodium hydroxide NaOH, 1 N

Procedure

1. One drop (0.05 mL) phenolpthalein was added to 50 mL of sample. If red color developed H_2SO_4 was added to discharge the color.
2. One mL H_2SO_4 and 0.5 g solid $K_2S_2O_8$ were then added.
3. The solution was boiled gently on a preheated hot plate for 30 to 40 mins or until 10 mL remained.
4. It was then cooled and diluted to 30 mL with distilled H_2O.
5. One drop (0.05 mL) phenolpthalein was added, followed by NaOH until the solution was faintly pink.
6. The solution was then made up to 100 mL with distilled water.

Phosphate analysis

The phosphorus in the sample solution was then determined by Methods 1 and 2.

Separate calibration graphs were constructed using phosphate standards that were also pretreated with persulfate.

Method 1:

Vanadomolybdophosphoric acid method

A wavelength of 470 nm was used. The yellow color was due to the molybdophosphoric acid in the presence of vanadium. The intensity of the yellow color from this complex formation was proportional to the phosphate concentration. Glassware was acid washed before use. The procedure is as described in reference 1.

Reagents

1. Phenolpthalein indicator solution
2. HCL solution (0.5 N)
3. Activated carbon
4. Vanadate-molybdate reagent

Solution A: 2.5 g of ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ was dissolved in 30.0 mL of distilled water.

Solution B: 0.125 g of ammonium metavanadate was dissolved by heating to boiling point in 30 mL of distilled water. It was cooled and 33 mL concentrated HCL was added.

Solution C: After cooling to room temperature, Solution A was mixed with Solution B, and diluted to 100 mL.

5. Standard phosphate solutions:

22.068 mg of anhydrous KH_2PO_4 was dissolved in distilled water and diluted to 100 mL. Thus, 1mL = 50.3µg PO_4^{3-}-P.

A series of standards were made: for example, 0.5 mL of stock was put into a 50 mL flask for standard 1, and 5 mLs of Solution C added. Distilled water was used to fill to the 50 mL mark. A calibration graph at 470 nm was obtained on the Cary 1 spectrophotometer using the Concentration Application. A 1 second signal averaging time (SAT) was used throughout.

Sample preparation

Procedures

1. If the digested sample pH was >10, 0.05 mL (1 drop) of phenopthalein was added to 50 mL sample. The pH was lowered with 0.5 N HCl before it was diluted to 100 mL.
2. 200 mg of activated carbon was then added to 50 mL of the sample. It was then shaken for 5 minutes in a flask then filtered to remove the carbon.
3. 35 mL of sample was placed into a 50 mL volumetric flask to which 10 mL of Solution C was added and diluted to the mark with distilled water.
4. The blank was 35 mL distilled water treated as in number 3, in another 50 mL volumetric flask.
5. After 10 minutes, the sample absorbance at 470 nm was measured and compared with that of the blank. Each of the calibration standards was read after a 10 minute delay.

AOAC has described Fe^{2+} and NO^{3-} as interferents. The sample was spiked with ferric nitrate to show the effects of an interferent (Figure 1). The concentration of ferric nitrate added was 27.83 mg/L.

The scan was compared with the unspiked sample scan. The absorbance readings at 470 nm were not affected by this interferent, as the ferrous and nitrate ion concentrations were still <100 and 1000 mg/L respectively, in the spiked sample.[1]

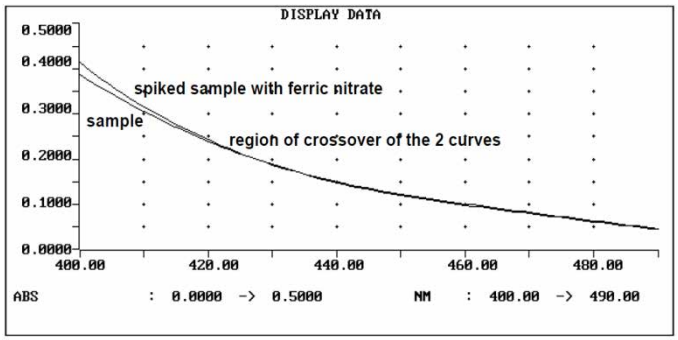

Figure 1. An example of an interferent $Fe(NO_3)_2$ spiking of hydroponics sample

A series of standards was made for calibration at 470 nm. The calibration graph obtained from this method is shown in Figure 2 below:

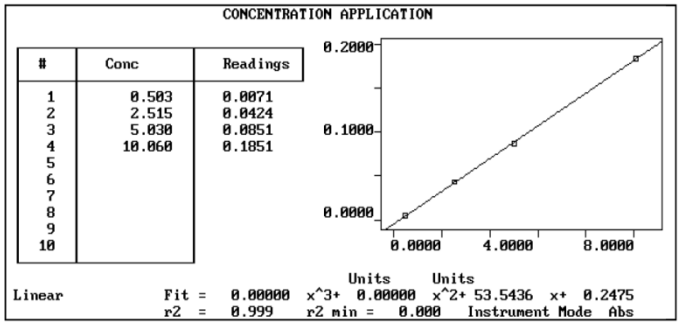

Figure 2. The calibration graph for method 1

The values obtained from the linear calibration graph were then corrected as in reference 1 for dilution factors.

The phosphorus concentration from the Absorbance at 470 nm of 0.08401 Abs was calculated as follows:

(4.745 µg/mL x (50 mL/35 mL))

= 6.78 mg/L

Method 2:

Stannous chloride method

Molybdophosphoric acid reduction by stannous chloride to molybdenum results in the development of a blue color. The procedure used was as described in reference 1. A wavelength of 690 nm was used.

Reagents

1. Phenolpthalein indicator solution
2. Strong acid solution: 300 mL conc H_2SO_4 was added slowly to 600 mL distilled water, cooled, then 4.0 mL conc HNO_3 was added before dilution to 1 L.

Procedure

1. Ammonium molybdate reagent 1: 2.5 g $(NH_4)_6Mo_7O_{24}.4H_2O$ was dissolved in 17.5 mL distilled water. 28 mL of conc H_2SO_4 was added to 40 mL distilled water. The molybdate was then added and diluted to 100 mL.
2. Stannous chloride reagent 1: 2.5 g of $SnCl_2.2H_2O$ was dissolved in 100 mL glycerol. Heat if necessary to aid dissolution.
3. Standard phosphate solutions (as in Method 1).

The samples, standards, and reagents were kept within 2 °C of one another and in the temperature range between 20 to 30 °C. Color measurements were taken 11 minutes after the reagents were added. The color was measured at 690 nm using distilled water as the blank. The light path used was 1 cm. No extraction[1] was required as the amount of phosphorus present in the solution was estimated from the results obtained in Method 1. Increased sensitivity is required only if the concentration is below 100 µg P/L.

The calibration graph for Method 2 is as shown in Figure 3.

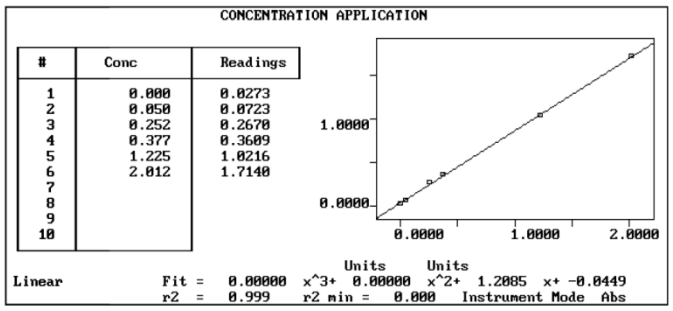

Figure 3. The calibration graph for method 2

Sample: Dilution factors

Forty milliliters of the sample was taken and diluted to 100 mL with distilled water after discharging the pink color with acid. Then 50 mL of this solution was taken and diluted to 100 mL with distilled water.

The calculations include the following dilution factors:

As in reference 1:

µg P/mL = value from line of best fit x 100/40 x 100/50

Thus, the phosphorus concentration measured from Method 2 (which gave an absorbance value of 1.2295 Abs at 690 nm) was calculated as follows:

1.4409 µg/mL x 2.5 x 2

= 7.2 µg/mL

or 7.2 mg/L

Discussion

The concentration of phosphorus in the hydroponics sample was found to be:

Method 1	Method 2
6.8 mg/L	7.2 mg/L

These results give an average of 7 mg/L of phosphorus in the hydroponics sample, and also correspond to the expected value for hydroponics sample. (See Appendix.)

The minimum detectable limit of phosphates using vanadomolybdophosphoric acid method is 200 µg P/L with a 1 cm pathlength cell, while the minimum detection limit using the stannous chloride is lower, about 3 µg P/L.[1] Thus, Method 2 is more sensitive than Method 1.

However, the color generated in Method 1 is stable and its intensity is unaffected by room temperature variations. In Method 2, the time taken to read both standards and samples was critical for the accuracy of the concentration readings.

The temperature of the reactions in Method 2 also affects the phosphate results. However, the choice of methods used to analyze phosphate quantitatively would depend on the interferents present and the detection limits required. The interferents and their levels affecting phosphate analyses are different for Methods 1 and 2.[1]

Increased sensitivity can be achieved in Method 2 by an extraction of molybdophosphoric acid into an organic solvent such as benzene-isobutanol. This additional procedure can improve the detection limit of this method to 3 µg P/L if required.

The concentration levels of various interferents are discussed elsewhere.[1] Clean acid washed glassware is also required for these analyses to prevent phosphate contamination.

Other reducing agents besides stannous chloride can be used to form the blue molybdenum compound. These include ascorbic acid, 1-amino-2-napthol-4 sulfuric acid, hydrazine sulfate and p-methylamino-phenol sulfate. However, stannous chloride (Method 1) and ascorbic acid (Method 3) are the most commonly used reducing agents.

In this hydroponics sample, both results are close, suggesting that both methods are suitable. The results also show that despite the presence of many ions in the sample, these ions have not affected the determination of phosphate. (See Appendix for the composition of the sample.)

These methods can be automated if required for greater sample throughput. Automation is possible when using the Cary 1/3. Automation can increase reliability as well as reproducibility by reducing operator errors that can become significant if too many samples are to be analyzed manually.

Conclusion

The phosphorus content in the hydroponics solution can be accurately measured using the Cary 1 and the Concentration Application software. No interference effects were observed using this methodology.

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Appendix

Table 1. Composition of hydroponics sample

Salts present in solution

KH_2PO_4
$Ca(NO_3)2.H_2O$
$MgSO_4.7H_2O$
KNO_3
K_2SO_4
$FeSO_4.7H_20$
$MnSO_4.4H_2O$
$ZnSO_4.7H_20$
$CuSO_4.5H_20$
$NH_4H_2PO_4$

Theoretical P content of sample

P in KH_2PO_4 = 5.7 mg/L
P in $NH_4H_2PO_4$ = 1.1 mg/L

Therefore:
P content in sample = 6.8 mg/L

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Agilent Technologies

Validation of a leaf reflectance and transmittance model for three agricultural crop species

Application Note

Author

G. J. Newnham* and T. Burt**

*Remote Sensing and Satellite Research Group, Curtin University of Technology, Perth, Western Australia

** Agilent Technologies, Inc., 679 Springvale Road, Mulgrave, Victoria 3170, Australia

Abstract

Reflectance and transmittance have been measured for a selection of leaves from three cereal crop species using a laboratory based Cary 500 spectrophotometer. Spectral measurements, along with ancillary data describing chlorophyll, water and dry matter content per unit leaf area, have been used to assess the accuracy of the Prospect model [1] for estimating leaf reflectance and transmittance. An inversion technique has also been applied to the model to derive leaf pigment and leaf water content. This has produced acceptable estimates of the true variation in these parameters. This result indicates that the Prospect model, in conjunction with a suitable method to account for canopy architecture and soil reflectance, has potential to contribute to the generation of crop status information products based on hyperspectral data.

Introduction

Increasingly, surface reflectance estimates derived from hyperspectral data are used to assess spatial variance in crop condition and biomass over large agricultural fields. The distribution and optical properties of individual plant leaves are the primary factors contributing to the reflectance of a mature crop canopy. Consequently, to develop valuable information products from these data sources, an understanding of the reflectance and transmittance of individual plant leaves is essential.

The Prospect leaf reflectance and transmittance model [1] has been developed using contemporary theories of radiative transfer within leaf material. However, the inherent variability in biochemical constituents and leaf structure among plant species requires that accurate laboratory validation of the model be undertaken before it can be used reliably for agricultural crop condition assessment.

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In this experiment, three cereal crop species (barley, oats and wheat) were chosen for analysis. Plants were sown in pots and placed in a greenhouse for four weeks prior to measurement. Etiolates were produced by placing selected plants in complete darkness over the final two weeks to inhibit chlorophyll synthesis. To examine the effect of variations in leaf water content, a selection of wheat leaves were picked 24 hours, 12 hours and 2 hours prior to measurement and placed in a warm oven set at 60 °C.

Healthy and etiolated leaves were picked from each plant an hour prior to spectrophotometric measurement. Replicates of each sample were selected and prepared for destructive analysis to derive values for the Prospect model input parameters; chlorophyll a and b, leaf water and leaf dry matter content per unit area of leaf matter.

Spectral measurements

The reflectance and transmittance of leaf samples were recorded over wavelengths from 350 nm to 2500 nm using a laboratory based Cary 500 spectrophotometer. The instrument was fitted with a 110 mm diameter integrating sphere, permitting the sum of direct and diffuse radiation to be the measured. A total of 28 spectra were recorded, constituting 14 pairs of reflectance and transmittance measurements.

Comparison between healthy and etiolated wheat leaf spectra shows the distinct impact on reflectance and transmittance due to pigment content. As shown in Figure 1, reflectance in the visible red region is reduced considerably in the etiolated leaves due to the inhibition of chlorophyll synthesis. The etiolated leaf spectra still exhibit a significant drop in response in the visible blue region due to the carotenoid group of pigments, which develop in leaves even in the absence of light [2].

The reflectance of a healthy wheat leaf also illustrates the major water absorption bands centered at 1450 and 1940 nm, easily distinguishable in fresh leaf spectra. However, the weaker liquid water absorption bands documented by [3] and centered at 760, 970, 1190, are more difficult to distinguish. The reflectance of a dry wheat leaf in Figure 1 shows a dramatic reduction in the near infrared. Remnant absorption featured across this region is due to biochemicals such as protein and cellulose. Chlorophyll absorption in these dry leaves remains strong due to the rapid nature of the drying process.

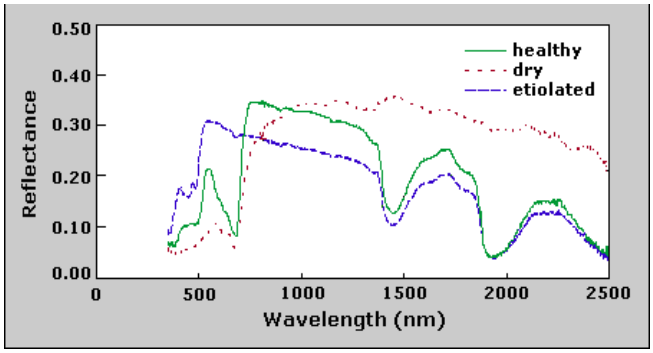

Figure 1. Reflectance spectra of healthy, dry and etiolated wheat leaves measured using the Cary 500 spectrophotometer

Variations in leaf thickness were simulated by stacking multiple leaves over the integrating sphere port. Leaf thickness contributes to the relative proportion of reflected versus transmitted light in each wavelength, altering the number of refractive index changes encountered in a direct path through the leaf, while also increasing the quantity of absorbing material present. This ratio of reflected versus transmitted light is highlighted in Figure 2, where the stacking of multiple leaves has increased the reflectance and decreased the transmittance, particularly in the low absorbing near infrared region.

Ancillary data

Three sets of absorption coefficients [1, 4, 5] have been defined for the Prospect model since its inception. Those used by [5] were selected for this study, as it was more practical to measure dry matter content than the specific biochemicals used by [4]. As a result, the model parameters used to compute leaf reflectance and transmittance comprised the content of chlorophyll, water and dry matter per unit leaf area, as well as a leaf structure parameter, to help define the amount of reflectance versus transmittance.

In order to determine chlorophyll content per unit leaf area, images of each leaf were recorded using a flat bed scanner and leaf area computed using digital image analysis. Each leaf was then ground in acetone to extract the leaf pigments and the solution filtered to remove undissolved leaf matter. Absorption of the dissolved pigment solution was measured using a spectrophotometer and normalized for solvent volume and leaf area. The content of chlorophyll *a* + *b* in the leaves was computed according to the method of [6] and recorded in units of $\mu g/cm^2$ of leaf area.

To derive leaf water and dry matter content, leaf area was again determined using digital image analysis. The fresh leaves were weighed and then placed in a warm oven set at 60 °C, for three days. When removed, a dry weight was recorded and this considered the true weight of dry matter in the fresh leaf. The difference between fresh and dry weights was assumed to account for the weight of water in the fresh leaf. Each weight was normalized for the respective leaf area and recorded in units of g/cm^2.

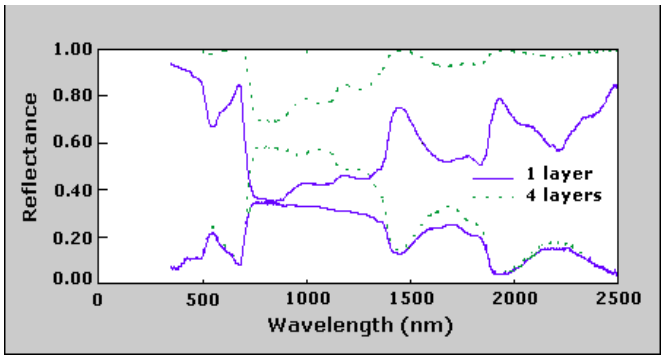

Figure 2. Reflectance (lower curves) and 1-transmittance (upper curves) measured for a single barley leaf layer (solid lines) and for a stack of four barley leaves (dotted lines)

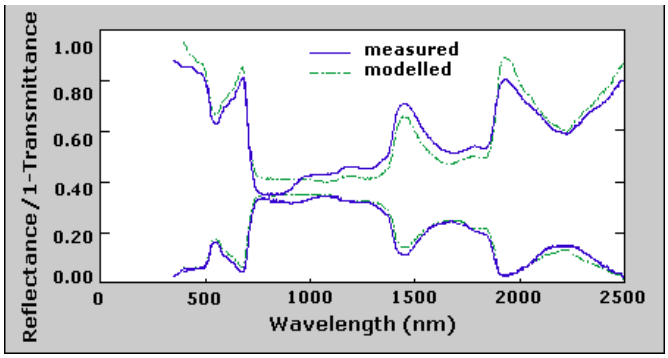

Figure 3. Graphical presentation of measured reflectance and 1-transmittance spectra (solid lines) and those produced using the Prospect model (dashed lines)

Model validation

Figure 3 shows a strong relationship between measured cereal crop leaf reflectance and transmittance spectra to those computed using the Prospect model. Correlation in the visible red chlorophyll absorbing region and the two major water absorption bands in the near infrared are particularly promising for inversion of the model.

Within the theoretical framework of the Prospect model, the leaf is considered to be a series of flat homogeneous plates. The relative proportion of light reflected and transmitted is accounted for by the structure parameter indicating the number of plates in the leaf structure, and the refractive index of these plates. The lowest value accepted by the model for this structure parameter is one. This was the value used for the computation of all spectra, except where multiple leaves were stacked over the integrating sphere port.

One apparent inconsistency between measured and modelled spectra is the general overestimation of reflectance and underestimation of transmittance. This is obvious in Figure 4, which shows the average modelling error for all measured and modelled spectra. This overestimation would be best addressed by reviewing the refractive index used for model calculations. It is assumed that lower refractive index values would allow a more meaningful structure parameter to be set and produce more accurate spectra.

One feature of the measured cereal crop leaf spectra that is not accounted for in the model is the dissimilarity between reflectance and transmittance spectra in the low absorption regions of the near infrared. A substantial increase in the transmittance centered in the region of 800 nm was seen in all fresh leaf spectra. However a corresponding increase is not displayed in the reflectance curves. This causes the underestimation of transmittance in this region seen in Figure 4. A possible reason for this feature is some form of anisotropic scattering causing a dominance of forward scattering. There is no easy way to account for wavelength dependent scattering features in the Prospect model other than to adjust the refractive index in this region. This is another motivation for investigating a possible underestimation of the refractive index in future work.

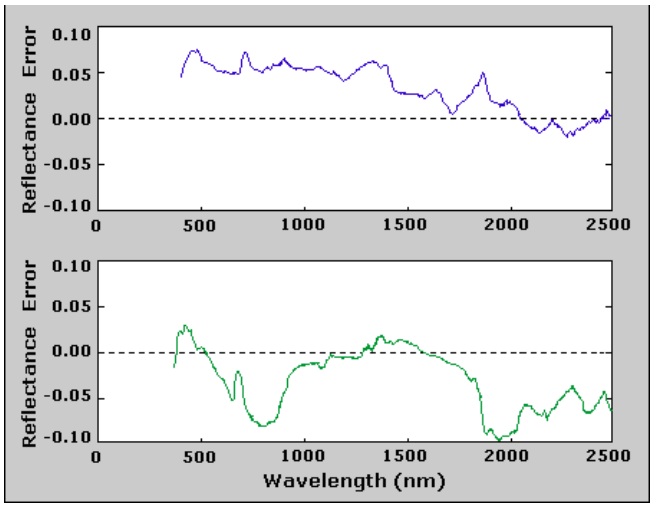

Figure 4. Average modelling error (modelled-measured) for the 14 reflectance and transmittance spectra

Model Inversion

The merit function shown below, defined by [1], was applied to the data to find a set of model parameters that minimize the difference between measured and modeled spectra. In this equation Δ^2 is the function to be minimized, $R_{Cary500}(\lambda)$ and $T_{Cary500}(\lambda)$ and the reflectance and transmittance at wavelength λ measured using the Cary 500, and $R_{Model}(\lambda)$ and $T_{Model}(\lambda)$ are the corresponding reflectance and transmittance estimated using the Prospect model.

$$\Delta^2 = \sum_{\lambda=400}^{2500} \left[\left(R_{Cary\,500}(\lambda) - R_{Model}(\lambda) \right)^2 + \left(T_{Cary\,500}(\lambda) - T_{Model}(\lambda) \right)^2 \right]$$

A generalized reduced gradient (GRG) constrained minimization routine [7] was used to minimise the merit function and derive estimates of leaf structure, leaf pigment, water and dry matter content. The values for chlorophyll and water were compared to measured values (Figure 5). Retrieval of leaf water content per unit leaf area was encouraging with only a slight overestimation shown by the best fit linear coefficient of 1.09824 and an R^2 of 0.9289. Chlorophyll content had a greater tendency for overestimation with a linear coefficient of 1.2399 and a reduced precision with an R^2 of 0.7846. Values retrieved from the inversion process versus their measured value are plotted in Figure 5.

Conclusions

The results of this work have shown that problems exist in the estimation of cereal crop leaf reflectance and transmittance using the Prospect model. This leads to inaccuracies in the retrieval of chlorophyll and leaf water content through model inversion. However, further work to adjust the leaf refractive index may help to address these problems and to achieve better estimates of these parameters.

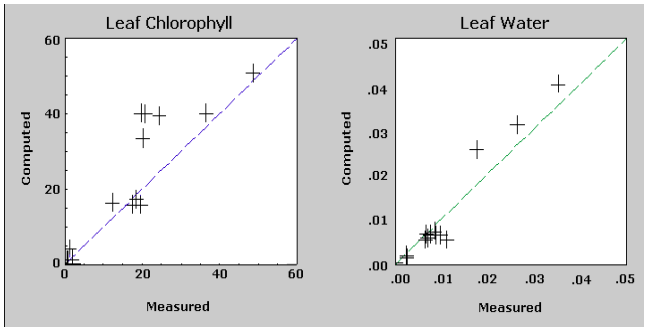

Figure 5. Values retrieved for leaf chlorophyll content and leaf water content plotted against their measured values, along with the line showing the ideal 1 to 1 relationship

With further work to identify its shortcomings specific to cereal crop analysis, the Prospect model, when coupled with a suitable canopy reflectance model, may

prove a valuable tool in the monitoring and management of agricultural crops using hyperspectral data.

Acknowledgments

The authors wish to thank Agilent Technologies Australia (M) Pty Ltd for the use of the Cary 500 spectrophotometer. We would also like to thank the Department of Environmental Biology at Curtin University of Technology for assistance in measuring ancillary data and Associate Professor Mervyn Lynch from the Department of Applied Physics for his technical assistance.

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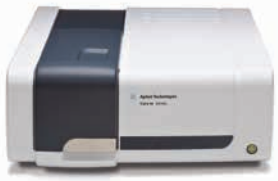

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Determination of phosphorus concentration in hydroponics solution

Application Note

Author

Sharon Lim

Agilent Technologies, Inc.
Mulgrave, Victoria 3170,
Australia

Introduction

Phosphates exist in many forms in water. They can occur as orthophosphates, condensed phosphates such as pyro, meta and polyphosphates or as organically bound phosphates.[1] The major pollution sources of phosphates in natural waters are detergents used in industry and fertilizers from agricultural farming.

The use of fertilizers is increasing. Phosphates in fertilizer and farm runoffs have to be monitored in order to prevent the possibility of polluting the surrounding environment. The excessive enrichment of natural waters with plant nutrients, known as eutrophication, can result in overabundant growth of undesirable algae and aquatic plants, affecting water quality.[2,3]

Solution spectrophotometry still remains an essential and reliable analytical method for routine water analysis, particularly for the determination of nutrient elements such as nitrogen and phosphorus.

Spectrophotometric methods often require the initial conversion of phosphates to soluble orthophosphates which can then be determined colorimetrically. Orthophosphates can be analyzed quantitatively by three methods:

1. Vanadomolybdophosphoric acid method
2. Stannous chloride method
3. Ascorbic acid method

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Methods 1 and 2 were used in this study to quantitate and confirm an unknown phosphorus concentration in a hydroponics solution. These methods are suitable for determining the level of phosphates such as KH_2PO_4 and $NH_4H_2PO_4$ added into fertilizers and hydroponics as sources of phosphorus. It is also important that the phosphate level conforms to plant requirements. If excess phosphates are used, this could lead to the death of plants as well as excess phosphate in runoffs.

Equipment

Instrument: Cary 1

Concentration Application software

Methodology

Digestion

Pretreatment of the hydroponics sample

The hydroponics sample was digested first, using the potassium persulfate method as described in reference 1. Acid digestion is necessary if total phosphorus is to be determined as phosphorus may be present in combination with organic matter.

Apparatus

1. Hot plate
2. Weighing boats

Reagents

1. Phenolpthalein indicator aqueous solution
2. Sulfuric acid solution (300 mL conc H_2SO_4 in 1 L H_2O)
3. Potassium persulfate $K_2S_2O_8$
4. Sodium hydroxide NaOH, 1 N

Procedure

1. One drop (0.05 mL) phenolpthalein was added to 50 mL of sample. If red color developed H_2SO_4 was added to discharge the color.
2. One mL H_2SO_4 and 0.5 g solid $K_2S_2O_8$ were then added.
3. The solution was boiled gently on a preheated hot plate for 30 to 40 mins or until 10 mL remained.
4. It was then cooled and diluted to 30 mL with distilled H_2O.
5. One drop (0.05 mL) phenolpthalein was added, followed by NaOH until the solution was faintly pink.
6. The solution was then made up to 100 mL with distilled water.

Phosphate analysis

The phosphorus in the sample solution was then determined by Methods 1 and 2.

Separate calibration graphs were constructed using phosphate standards that were also pretreated with persulfate.

Method 1:

Vanadomolybdophosphoric acid method

A wavelength of 470 nm was used. The yellow color was due to the molybdophosphoric acid in the presence of vanadium. The intensity of the yellow color from this complex formation was proportional to the phosphate concentration. Glassware was acid washed before use. The procedure is as described in reference 1.

Reagents

1. Phenolpthalein indicator solution
2. HCL solution (0.5 N)
3. Activated carbon
4. Vanadate-molybdate reagent

Solution A: 2.5 g of ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ was dissolved in 30.0 mL of distilled water.

Solution B: 0.125 g of ammonium metavanadate was dissolved by heating to boiling point in 30 mL of distilled water. It was cooled and 33 mL concentrated HCL was added.

Solution C: After cooling to room temperature, Solution A was mixed with Solution B, and diluted to 100 mL.

5. Standard phosphate solutions:

22.068 mg of anhydrous KH_2PO_4 was dissolved in distilled water and diluted to 100 mL. Thus, 1mL = 50.3µg PO_4^{3-}-P.

A series of standards were made: for example, 0.5 mL of stock was put into a 50 mL flask for standard 1, and 5 mLs of Solution C added. Distilled water was used to fill to the 50 mL mark. A calibration graph at 470 nm was obtained on the Cary 1 spectrophotometer using the Concentration Application. A 1 second signal averaging time (SAT) was used throughout.

Sample preparation

Procedures

1. If the digested sample pH was >10, 0.05 mL (1 drop) of phenopthalein was added to 50 mL sample. The pH was lowered with 0.5 N HCl before it was diluted to 100 mL.
2. 200 mg of activated carbon was then added to 50 mL of the sample. It was then shaken for 5 minutes in a flask then filtered to remove the carbon.
3. 35 mL of sample was placed into a 50 mL volumetric flask to which 10 mL of Solution C was added and diluted to the mark with distilled water.
4. The blank was 35 mL distilled water treated as in number 3, in another 50 mL volumetric flask.
5. After 10 minutes, the sample absorbance at 470 nm was measured and compared with that of the blank. Each of the calibration standards was read after a 10 minute delay.

AOAC has described Fe^{2+} and NO^{3-} as interferents. The sample was spiked with ferric nitrate to show the effects of an interferent (Figure 1). The concentration of ferric nitrate added was 27.83 mg/L.

The scan was compared with the unspiked sample scan. The absorbance readings at 470 nm were not affected by this interferent, as the ferrous and nitrate ion concentrations were still <100 and 1000 mg/L respectively, in the spiked sample.[1]

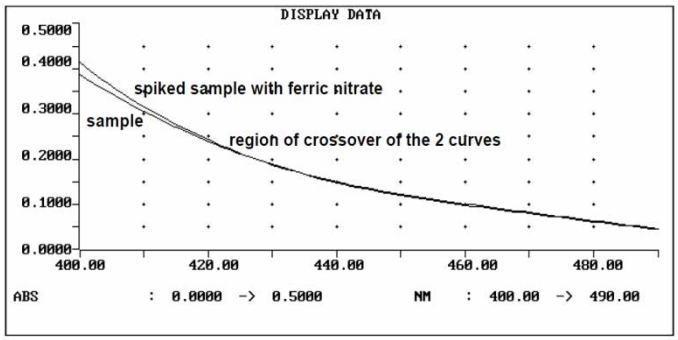

Figure 1. An example of an interferent $Fe(NO_3)_2$ spiking of hydroponics sample

A series of standards was made for calibration at 470 nm. The calibration graph obtained from this method is shown in Figure 2 below:

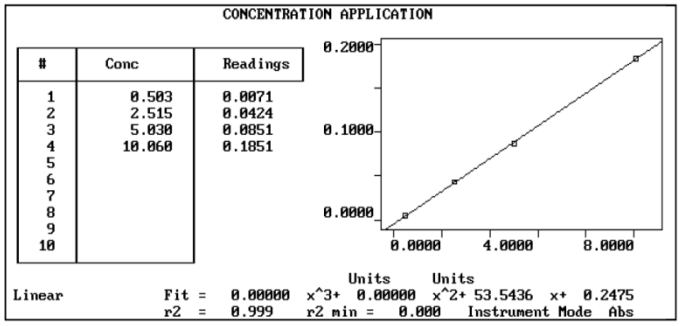

Figure 2. The calibration graph for method 1

The values obtained from the linear calibration graph were then corrected as in reference 1 for dilution factors.

The phosphorus concentration from the Absorbance at 470 nm of 0.08401 Abs was calculated as follows:

(4.745 µg/mL x (50 mL/35 mL))

= 6.78 mg/L

Method 2:

Stannous chloride method

Molybdophosphoric acid reduction by stannous chloride to molybdenum results in the development of a blue color. The procedure used was as described in reference 1. A wavelength of 690 nm was used.

Reagents

1. Phenolpthalein indicator solution
2. Strong acid solution: 300 mL conc H_2SO_4 was added slowly to 600 mL distilled water, cooled, then 4.0 mL conc HNO_3 was added before dilution to 1 L.

Procedure

1. Ammonium molybdate reagent 1: 2.5 g $(NH_4)_6Mo_7O_{24}.4H_2O$ was dissolved in 17.5 mL distilled water. 28 mL of conc H_2SO_4 was added to 40 mL distilled water. The molybdate was then added and diluted to 100 mL.
2. Stannous chloride reagent 1: 2.5 g of $SnCl_2.2H_2O$ was dissolved in 100 mL glycerol. Heat if necessary to aid dissolution.
3. Standard phosphate solutions (as in Method 1).

The samples, standards, and reagents were kept within 2 °C of one another and in the temperature range between 20 to 30 °C. Color measurements were taken 11 minutes after the reagents were added. The color was measured at 690 nm using distilled water as the blank. The light path used was 1 cm. No extraction[1] was required as the amount of phosphorus present in the solution was estimated from the results obtained in Method 1. Increased sensitivity is required only if the concentration is below 100 µg P/L.

The calibration graph for Method 2 is as shown in Figure 3.

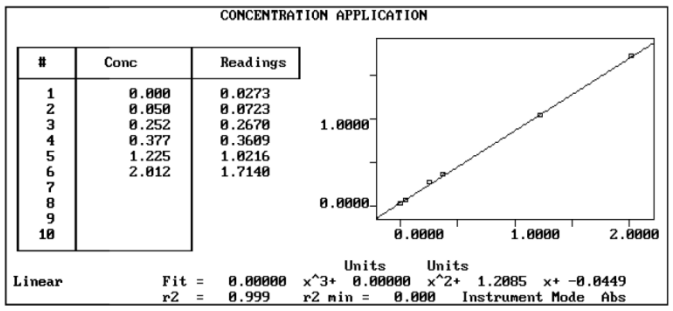

Figure 3. The calibration graph for method 2

Sample: Dilution factors

Forty milliliters of the sample was taken and diluted to 100 mL with distilled water after discharging the pink color with acid. Then 50 mL of this solution was taken and diluted to 100 mL with distilled water.

The calculations include the following dilution factors:

As in reference 1:

µg P/mL = value from line of best fit x 100/40 x 100/50

Thus, the phosphorus concentration measured from Method 2 (which gave an absorbance value of 1.2295 Abs at 690 nm) was calculated as follows:

1.4409 µg/mL x 2.5 x 2

= 7.2 µg/mL

or 7.2 mg/L

Discussion

The concentration of phosphorus in the hydroponics sample was found to be:

Method 1	Method 2
6.8 mg/L	7.2 mg/L

These results give an average of 7 mg/L of phosphorus in the hydroponics sample, and also correspond to the expected value for hydroponics sample. (See Appendix.)

The minimum detectable limit of phosphates using vanadomolybdophosphoric acid method is 200 µg P/L with a 1 cm pathlength cell, while the minimum detection limit using the stannous chloride is lower, about 3 µg P/L.[1] Thus, Method 2 is more sensitive than Method 1.

However, the color generated in Method 1 is stable and its intensity is unaffected by room temperature variations. In Method 2, the time taken to read both standards and samples was critical for the accuracy of the concentration readings.

The temperature of the reactions in Method 2 also affects the phosphate results. However, the choice of methods used to analyze phosphate quantitatively would depend on the interferents present and the detection limits required. The interferents and their levels affecting phosphate analyses are different for Methods 1 and 2.[1]

Increased sensitivity can be achieved in Method 2 by an extraction of molybdophosphoric acid into an organic solvent such as benzene-isobutanol. This additional procedure can improve the detection limit of this method to 3 µg P/L if required.

The concentration levels of various interferents are discussed elsewhere.[1] Clean acid washed glassware is also required for these analyses to prevent phosphate contamination.

Other reducing agents besides stannous chloride can be used to form the blue molybdenum compound. These include ascorbic acid, 1-amino-2-napthol-4 sulfuric acid, hydrazine sulfate and p-methylamino-phenol sulfate. However, stannous chloride (Method 1) and ascorbic acid (Method 3) are the most commonly used reducing agents.

In this hydroponics sample, both results are close, suggesting that both methods are suitable. The results also show that despite the presence of many ions in the sample, these ions have not affected the determination of phosphate. (See Appendix for the composition of the sample.)

These methods can be automated if required for greater sample throughput. Automation is possible when using the Cary 1/3. Automation can increase reliability as well as reproducibility by reducing operator errors that can become significant if too many samples are to be analyzed manually.

Conclusion

The phosphorus content in the hydroponics solution can be accurately measured using the Cary 1 and the Concentration Application software. No interference effects were observed using this methodology.

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Appendix

Table 1. Composition of hydroponics sample

Salts present in solution

KH_2PO_4
$Ca(NO_3)2.H_2O$
$MgSO_4.7H_2O$
KNO_3
K_2SO_4
$FeSO_4.7H_20$
$MnSO_4.4H_2O$
$ZnSO_4.7H_20$
$CuSO_4.5H_20$
$NH_4H_2PO_4$

Theoretical P content of sample

P in KH_2PO_4 = 5.7 mg/L
P in $NH_4H_2PO_4$ = 1.1 mg/L

Therefore:
P content in sample = 6.8 mg/L

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Validation of a leaf reflectance and transmittance model for three agricultural crop species

Application Note

Author

G. J. Newnham* and T. Burt**

*Remote Sensing and Satellite Research Group, Curtin University of Technology, Perth, Western Australia

** Agilent Technologies, Inc., 679 Springvale Road, Mulgrave, Victoria 3170, Australia

Abstract

Reflectance and transmittance have been measured for a selection of leaves from three cereal crop species using a laboratory based Cary 500 spectrophotometer. Spectral measurements, along with ancillary data describing chlorophyll, water and dry matter content per unit leaf area, have been used to assess the accuracy of the Prospect model [1] for estimating leaf reflectance and transmittance. An inversion technique has also been applied to the model to derive leaf pigment and leaf water content. This has produced acceptable estimates of the true variation in these parameters. This result indicates that the Prospect model, in conjunction with a suitable method to account for canopy architecture and soil reflectance, has potential to contribute to the generation of crop status information products based on hyperspectral data.

Introduction

Increasingly, surface reflectance estimates derived from hyperspectral data are used to assess spatial variance in crop condition and biomass over large agricultural fields. The distribution and optical properties of individual plant leaves are the primary factors contributing to the reflectance of a mature crop canopy. Consequently, to develop valuable information products from these data sources, an understanding of the reflectance and transmittance of individual plant leaves is essential.

The Prospect leaf reflectance and transmittance model [1] has been developed using contemporary theories of radiative transfer within leaf material. However, the inherent variability in biochemical constituents and leaf structure among plant species requires that accurate laboratory validation of the model be undertaken before it can be used reliably for agricultural crop condition assessment.

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In this experiment, three cereal crop species (barley, oats and wheat) were chosen for analysis. Plants were sown in pots and placed in a greenhouse for four weeks prior to measurement. Etiolates were produced by placing selected plants in complete darkness over the final two weeks to inhibit chlorophyll synthesis. To examine the effect of variations in leaf water content, a selection of wheat leaves were picked 24 hours, 12 hours and 2 hours prior to measurement and placed in a warm oven set at 60 °C.

Healthy and etiolated leaves were picked from each plant an hour prior to spectrophotometric measurement. Replicates of each sample were selected and prepared for destructive analysis to derive values for the Prospect model input parameters; chlorophyll a and b, leaf water and leaf dry matter content per unit area of leaf matter.

Spectral measurements

The reflectance and transmittance of leaf samples were recorded over wavelengths from 350 nm to 2500 nm using a laboratory based Cary 500 spectrophotometer. The instrument was fitted with a 110 mm diameter integrating sphere, permitting the sum of direct and diffuse radiation to be the measured. A total of 28 spectra were recorded, constituting 14 pairs of reflectance and transmittance measurements.

Comparison between healthy and etiolated wheat leaf spectra shows the distinct impact on reflectance and transmittance due to pigment content. As shown in Figure 1, reflectance in the visible red region is reduced considerably in the etiolated leaves due to the inhibition of chlorophyll synthesis. The etiolated leaf spectra still exhibit a significant drop in response in the visible blue region due to the carotenoid group of pigments, which develop in leaves even in the absence of light [2].

The reflectance of a healthy wheat leaf also illustrates the major water absorption bands centered at 1450 and 1940 nm, easily distinguishable in fresh leaf spectra. However, the weaker liquid water absorption bands documented by [3] and centered at 760, 970, 1190, are more difficult to distinguish. The reflectance of a dry wheat leaf in Figure 1 shows a dramatic reduction in the near infrared. Remnant absorption featured across this region is due to biochemicals such as protein and cellulose. Chlorophyll absorption in these dry leaves remains strong due to the rapid nature of the drying process.

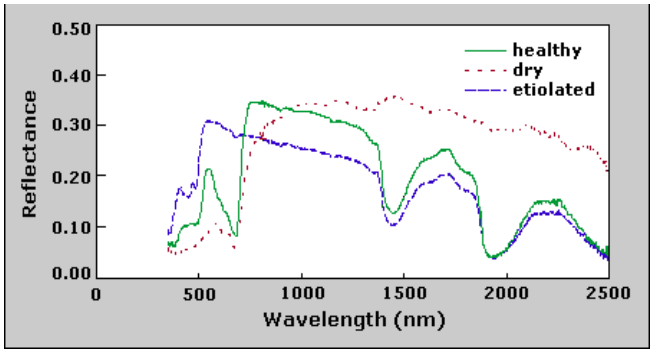

Figure 1. Reflectance spectra of healthy, dry and etiolated wheat leaves measured using the Cary 500 spectrophotometer

Variations in leaf thickness were simulated by stacking multiple leaves over the integrating sphere port. Leaf thickness contributes to the relative proportion of reflected versus transmitted light in each wavelength, altering the number of refractive index changes encountered in a direct path through the leaf, while also increasing the quantity of absorbing material present. This ratio of reflected versus transmitted light is highlighted in Figure 2, where the stacking of multiple leaves has increased the reflectance and decreased the transmittance, particularly in the low absorbing near infrared region.

Ancillary data

Three sets of absorption coefficients [1, 4, 5] have been defined for the Prospect model since its inception. Those used by [5] were selected for this study, as it was more practical to measure dry matter content than the specific biochemicals used by [4]. As a result, the model parameters used to compute leaf reflectance and transmittance comprised the content of chlorophyll, water and dry matter per unit leaf area, as well as a leaf structure parameter, to help define the amount of reflectance versus transmittance.

In order to determine chlorophyll content per unit leaf area, images of each leaf were recorded using a flat bed scanner and leaf area computed using digital image analysis. Each leaf was then ground in acetone to extract the leaf pigments and the solution filtered to remove undissolved leaf matter. Absorption of the dissolved pigment solution was measured using a spectrophotometer and normalized for solvent volume and leaf area. The content of chlorophyll *a* + *b* in the leaves was computed according to the method of [6] and recorded in units of $\mu g/cm^2$ of leaf area.

To derive leaf water and dry matter content, leaf area was again determined using digital image analysis. The fresh leaves were weighed and then placed in a warm oven set at 60 °C, for three days. When removed, a dry weight was recorded and this considered the true weight of dry matter in the fresh leaf. The difference between fresh and dry weights was assumed to account for the weight of water in the fresh leaf. Each weight was normalized for the respective leaf area and recorded in units of g/cm^2.

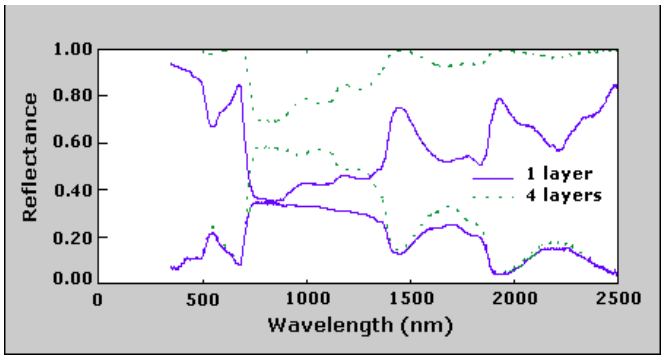

Figure 2. Reflectance (lower curves) and 1-transmittance (upper curves) measured for a single barley leaf layer (solid lines) and for a stack of four barley leaves (dotted lines)

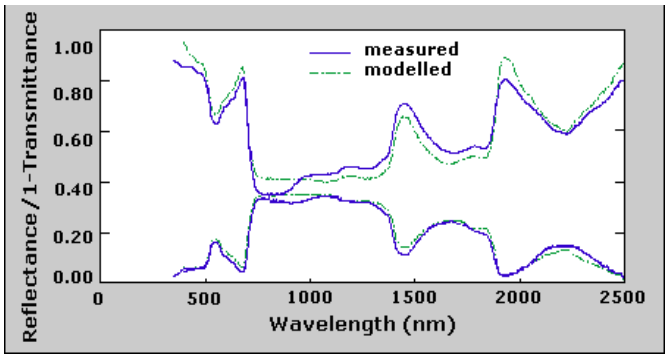

Figure 3. Graphical presentation of measured reflectance and 1-transmittance spectra (solid lines) and those produced using the Prospect model (dashed lines)

Model validation

Figure 3 shows a strong relationship between measured cereal crop leaf reflectance and transmittance spectra to those computed using the Prospect model. Correlation in the visible red chlorophyll absorbing region and the two major water absorption bands in the near infrared are particularly promising for inversion of the model.

Within the theoretical framework of the Prospect model, the leaf is considered to be a series of flat homogeneous plates. The relative proportion of light reflected and transmitted is accounted for by the structure parameter indicating the number of plates in the leaf structure, and the refractive index of these plates. The lowest value accepted by the model for this structure parameter is one. This was the value used for the computation of all spectra, except where multiple leaves were stacked over the integrating sphere port.

One apparent inconsistency between measured and modelled spectra is the general overestimation of reflectance and underestimation of transmittance. This is obvious in Figure 4, which shows the average modelling error for all measured and modelled spectra. This overestimation would be best addressed by reviewing the refractive index used for model calculations. It is assumed that lower refractive index values would allow a more meaningful structure parameter to be set and produce more accurate spectra.

One feature of the measured cereal crop leaf spectra that is not accounted for in the model is the dissimilarity between reflectance and transmittance spectra in the low absorption regions of the near infrared. A substantial increase in the transmittance centered in the region of 800 nm was seen in all fresh leaf spectra. However a corresponding increase is not displayed in the reflectance curves. This causes the underestimation of transmittance in this region seen in Figure 4. A possible reason for this feature is some form of anisotropic scattering causing a dominance of forward scattering. There is no easy way to account for wavelength dependent scattering features in the Prospect model other than to adjust the refractive index in this region. This is another motivation for investigating a possible underestimation of the refractive index in future work.

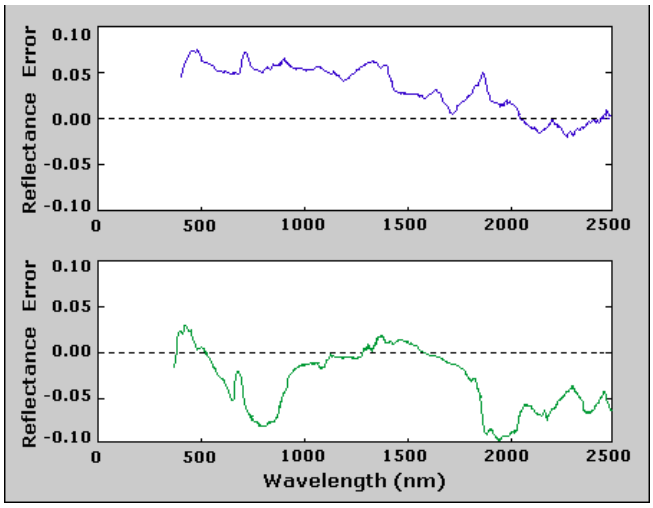

Figure 4. Average modelling error (modelled-measured) for the 14 reflectance and transmittance spectra

Model Inversion

The merit function shown below, defined by [1], was applied to the data to find a set of model parameters that minimize the difference between measured and modeled spectra. In this equation Δ^2 is the function to be minimized, $R_{Cary500}(\lambda)$ and $T_{Cary500}(\lambda)$ and the reflectance and transmittance at wavelength λ measured using the Cary 500, and $R_{Model}(\lambda)$ and $T_{Model}(\lambda)$ are the corresponding reflectance and transmittance estimated using the Prospect model.

$$\Delta^2 = \sum_{\lambda=400}^{2500} \left[\left(R_{Cary\,500}(\lambda) - R_{Model}(\lambda) \right)^2 + \left(T_{Cary\,500}(\lambda) - T_{Model}(\lambda) \right)^2 \right]$$

A generalized reduced gradient (GRG) constrained minimization routine [7] was used to minimise the merit function and derive estimates of leaf structure, leaf pigment, water and dry matter content. The values for chlorophyll and water were compared to measured values (Figure 5). Retrieval of leaf water content per unit leaf area was encouraging with only a slight overestimation shown by the best fit linear coefficient of 1.09824 and an R^2 of 0.9289. Chlorophyll content had a greater tendency for overestimation with a linear coefficient of 1.2399 and a reduced precision with an R^2 of 0.7846. Values retrieved from the inversion process versus their measured value are plotted in Figure 5.

Conclusions

The results of this work have shown that problems exist in the estimation of cereal crop leaf reflectance and transmittance using the Prospect model. This leads to inaccuracies in the retrieval of chlorophyll and leaf water content through model inversion. However, further work to adjust the leaf refractive index may help to address these problems and to achieve better estimates of these parameters.

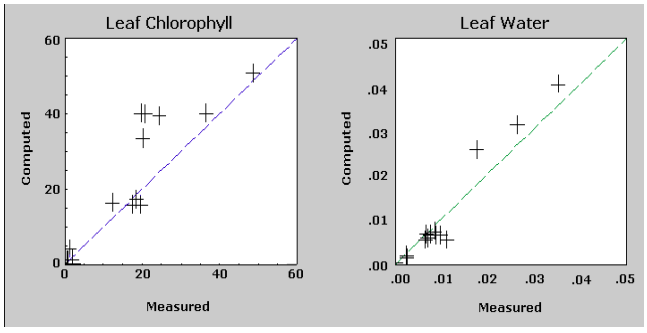

Figure 5. Values retrieved for leaf chlorophyll content and leaf water content plotted against their measured values, along with the line showing the ideal 1 to 1 relationship

With further work to identify its shortcomings specific to cereal crop analysis, the Prospect model, when coupled with a suitable canopy reflectance model, may

prove a valuable tool in the monitoring and management of agricultural crops using hyperspectral data.

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