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APPLICATION NOTE

GOT MILK FAT? USING NIR SPECTROSCOPY TO DETECT FAT CONTENT IN MILK

INTRO
BACKGROUND OF APPLICATION

Today's manufacturing environment requires high throughput, non-contact, non-destructive testing of a broad range of foodstuffs throughout the production process; as the food products industry relies on a global supply chain. Near-infrared (NIR) spectroscopy offers an optimal solution for inspecting incoming raw materials, analyzing composition, optimizing processes, and detecting fraudulent activities. By utilizing low-energy light to identify slight vibrations in molecules, NIR can effortlessly detect parameters such as water, proteins, and fats. With high-speed, non-destructive capabilities, NIR instruments are capable of conducting confirmatory testing of food product composition, providing near-instantaneous results that often match the accuracy of laboratory tests. NIR spectroscopy is particularly interesting in the dairy industry, where the percent fat content is often highly regulated. For example, in the United States, federal law requires that all butter contain at least 80% butterfat, French law requires 82% or greater butterfat, and the European Union places an upper limit on the amount of butterfat at 90%. Similar regulations exist governing milk; for example, to be marketed as whole milk it must contain a minimum of 3.25% milk fat, and to be marked as skim milk it must contain less than 0.15% milk fat.

In the NIR region, there exist multiple absorption bands for water and fat, but the 925 nm fat band and the 975 nm water band are especially intriguing to the dairy industry since they are detectable by silicon-based detectors. While this dramatically simplifies the cost and complexity of the hardware setup, it leads to additional complications concerning spectral interpretation. Since water molecules are highly polar and fat molecules are extremely non-polar, the water band is far more absorptive compared to the fat band. As a result, even though they are 50 nm apart, the water band can completely overpower the fat band at high water concentrations, requiring chemometric analysis. Fortunately, there are off-the-shelf commercially available software packages specifically designed for these types of applications. In this application note, we will examine a relatively simple use case by developing a regression model to differentiate between whole, skim, and reduced fat (2%) milk from two different manufacturers, demonstrating the ability to predict fat content in the presence of an overwhelming water absorption signal.

FIGURE 1 Milk Samples

For this experiment, we used two different brands of milk, hereby known as Milk-A and Milk-B. For simplicity, we chose ultra-filtered milk, which is a process where milk is pressurized and then run through a thin membrane to separate the water and the lactose from the rest of the milk. This process reduces the chemical complexity and the number of particulates in the mixture, helping to mitigate scattering effects. Based on the self-reported nutrition facts, we calculated the fat and protein concentrations for all six milk samples – two whole, two skim, and two reduced fat. As shown in Figure 1 we determined that whole Milk-A was 3.3% fat and 3.3% protein, whole Milk-B was 3.3% fat and 5.4% protein, reduced fat Milk-A was 2.1% fat and 3.3% protein, reduced fat Milk-B was 1.9% fat and 5.4% protein, skim Milk-A was 0% fat and 3.3% protein, and skim Milk-B was 0% fat and 5.4% protein. It is important to note that to develop a truly accurate analytical method, far more samples with far greater variability would be needed. Additionally, it would be necessary to use a confirmatory technique to validate the exact fat, protein, and water content. But, since the goal of this experiment was simply to show the method's applicability, we did not find the additional effort worthwhile.

EXPERIMENT SETUP

Due to the high optical density of the milk samples, we chose a reflective measurement approach, utilizing the Avantes FCR-7UVIR400-2-ME fiber coupled reflectance probe. The FCR-7UVIR400-2-ME has a 6-1 bifurcated cable design, with an outer ring of six illumination fibers and a central collection fiber. This design allows for dark field collection, significantly reducing the specular reflection while allowing a highly efficient 180o collection geometry. The bifurcated leg containing the six illumination fibers was connected to the Avantes AvaLight-HAL-S-Mini fiber-coupled constant current tungsten halogen light source. The AvaLight-HAL-S-Mini features an internal TTL-shutter, which provides the ability to periodically block the light source to take dark spectra during the experiment. The collection fiber was connected to an Avantes AvaSpec-ULS2048XL-EVO high-sensitivity back-thinned CCD spectrometer with a 600 – 1000 nm spectral range. Unlike many back-thinned CCD spectrometers, which have two-dimensional arrays, the ULS2048XL-EVO has large monolithic pixels of 14 X 500 square microns providing exceptional quantum efficiency in the NIR. All samples were measured at 21o C using a 5.5 ms integration time with 15 averages. Twenty spectra were collected for each sample, resulting in a total training set of 1200 spectra.

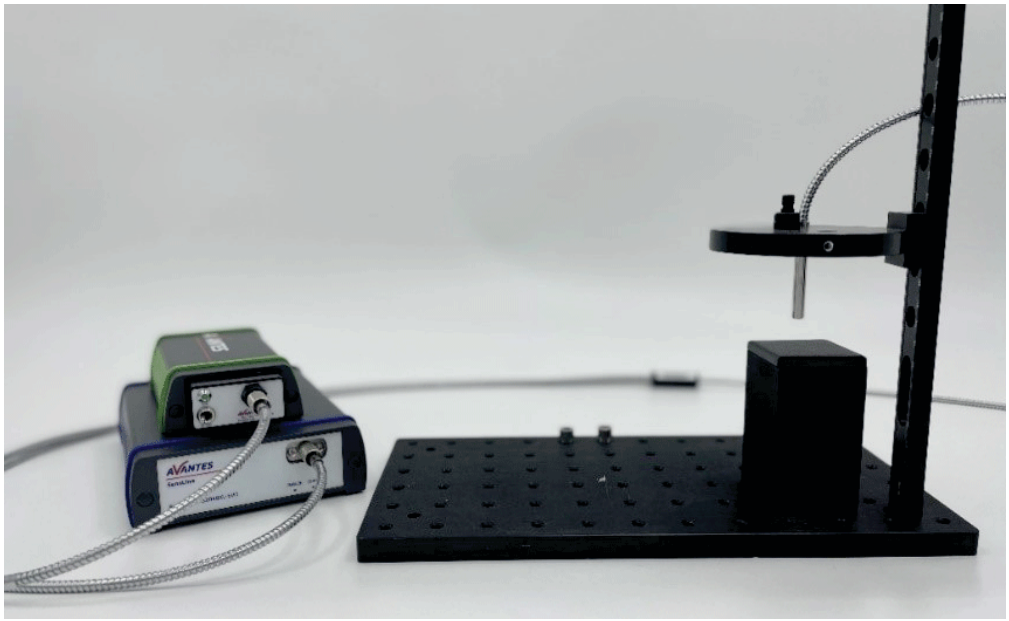

FIGURE 2 Experiment Setup

All spectra were collected and analyzed using the Panorama software package from LabCognition. Panorama offers a wide range of spectral processing, including baseline correction, normalization, and multiple derivatives, as well as, univariate and chemometric analysis tools for classification and regression. For this experiment, we used the Calibration Wizard feature in Panorama, which helps guide users through the process of determining which spectral processing and statistical analysis tools are best for a given application. Using the wizard, we chose the type-1 partial least squares regression model (PLS1) with a single dependent variable, fat concentration. The model was developed using the full spectra range, and the data was pretreated using mean centering.

RESULTS AND DISCUSSION

Based on the raw spectra (see Figure 3), we can see a clear trend in the NIR absorbance spectra. The two skim milk samples (Milk-B purple and Milk-A burgundy) are the most absorptive, the two reduced fat samples (Milk-B green and Milk-A yellow) are less absorptive, and the whole milk samples (Milk-B black and Milk-A pink) are the least absorptive; which is in perfect alignment with the increased absorption with increased water content. Furthermore, we can see that in all cases, Milk-B, which has a higher protein content, is slightly more absorptive than Milk-A. While there are no direct N-H (protein) absorption bands below 1000nm, the additional C-H and O-H bonds within the protein could be contributing to the increased absorption. Additionally, the excess protein's presence may alter the sample's scattering profile, leading to an apparent increase in absorption.

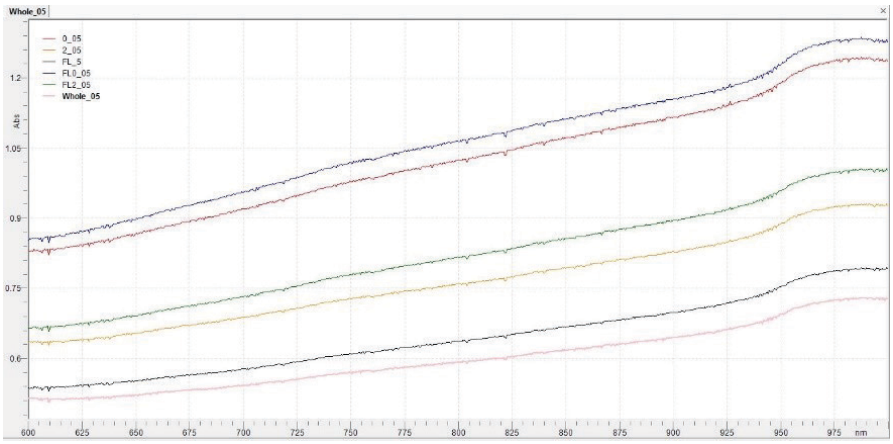

FIGURE 3: Representative raw Spectra of all six milk samples

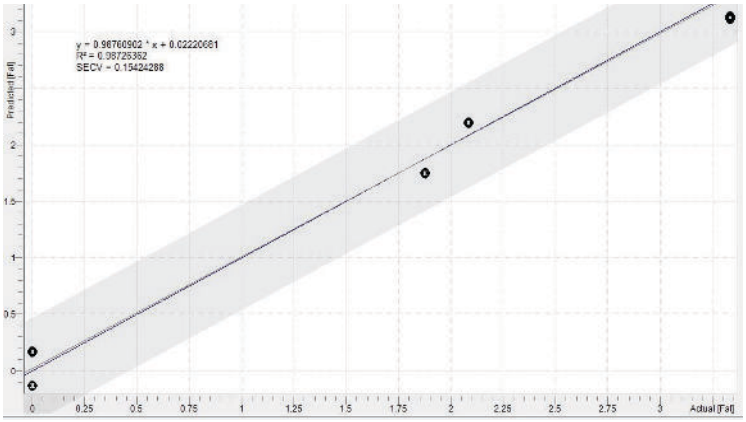

FIGURE 4: PLS1 Predictive Fat Content

After creating the PLS1 regression model in Panorama (Figure 4), we produced a linear calibration curve with predicted milk fat content on the y-axis and actual milk fat content on the x-axis. Not only is this calibration curve extremely linear (R2 = 0.9876362) with a standard error of cross-validation (SECV) of 0.1542488. This means that using this straightforward model built from four different milk fat concentrations, we were able to accurately predict milk fat content to within ~0.15%, which is more than applicable for most dairy applications. In fact, as discussed previously, in the United States, fat content below 0.15% can legally be self-reported as fat-free. Therefore, our observed margin of error is well within the expected error range of the reported nutrition facts. As previously discussed, if a higher degree of accuracy is required, this can be easily accomplished by using a wider range of fat concentrations in the training set and using an independent validation method to not rely on self-reported milk fat values.

Lastly, as an additional confirmation that the model is actually detecting variations in the fat band at 925 nm, we examined the residual plot generated in Panorama. Looking at the residuals across the entire spectrum allows us to see which spectral regions have high variance within the model. Based on these results, we can clearly see the influence of the 925 nm band across multiple samples, and the water absorption band does not overshadow it at 975 nm like it was in the raw spectra. It should be noted that since the spectra were all mean-centered, the contribution from the residual spectra should be viewed from the perspective of the absolute value or the deviation from the zero line. Therefore, the inverted peaks are functionally no different from the more traditional-looking peaks.

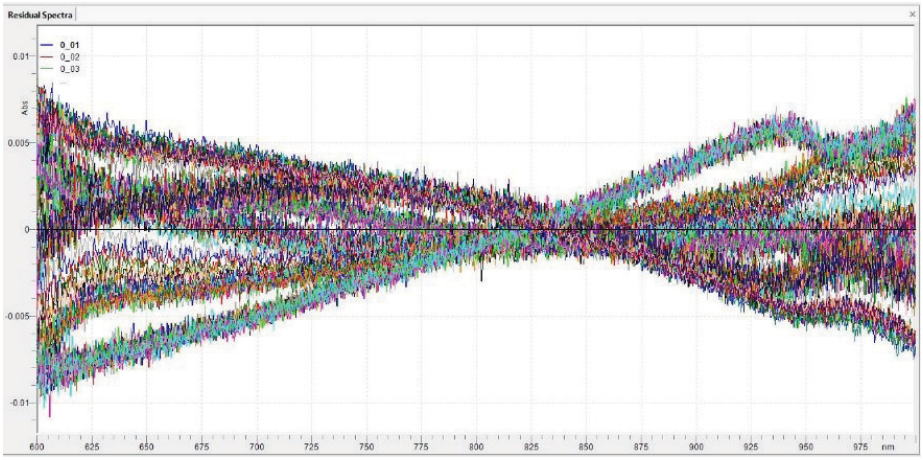

FIGURE 5: Residuals for Fat content of all six samples

FINAL THOUGHTS

While milk fat content was the focus of this application note, it is essential to remember that NIR spectroscopy and chemometric analysis can be applied to a wide variety of processes, laboratory, and field applications. In addition to the high-efficiency silicon-based detector utilized in this experiment, Avantes also offers a wide range of spectrometers that use InGaAs-based sensors capable of detecting light out to 2500 nm. This provides access to a more extensive range of chemical absorption bands and facilitates the analysis of materials ranging from complex polymers to simple glass molecules.

It is also important to note that while all the components used were standalone modules, they are also available as OEM modules or can be integrated into multichannel rack mount systems. These units can communicate via USB, Ethernet, and the native digital & analog input/output capabilities of the Avantes AS7010 electronics board provides for a superior interface with other devices. Additionally, the Avantes AvaSpec DLL package, with sample programs in Delphi, Visual Basic, C#, C++, LabView, MatLab, Python and many other programming environments, enables users to develop their own code.

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Avantes Headquarters
Phone: +31 (0) 313 670 170
Email: info@avantes.com
Website: www.avantes.com

Avantes Inc.
Phone: +1 (303) 410 866 8
Email: infousa@avantes.com
Website: www.avantesUSA.com

Avantes China
Phone: +86 (0) 108 457 404 5
Email: info@avantes.com.cn
Website: www.avantes.cn

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