Application note

Prediction of choline chloride concentration on silage

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Measuring the amount of choline chloride on animal feed is vital to the livestock industry. Choline chloride is sprayed on ground corncob (silage) as an added vitamin that helps animal growth and promotes health. Current methods for analyzing the amount of choline chloride are tedious and time-consuming (up to 12 hours per sample). Analysis using the Thermo Scientific™ Antaris™ FT-NIR Analyzer successfully measured choline chloride content on production samples directly through a plastic bag in less than 6 seconds without sacrificing accuracy or precision.

Introduction

Choline chloride (ChCl, chemical name trimethyl (2-hydroxyethyl) ammonium chloride) is an animal feed additive, classified as a water-soluble B-vitamin that increases animal growth. ChCl is added exogenously to feed stocks because it plays an essential role in fat transport and metabolism, while also protecting cell membrane structure. Typically, silage is used for ease of ingestion.

Currently, the most common ways to measure choline chloride content on silage are the Volhard method, perchloric acid titration, reaction with sodium tetraphenylborate, and a Reinke salt color comparison. All of these methods require soaking the ground corncob in solvent for 6 –12 hours to extract the choline chloride, followed by filtration concentration, and finally titration. The measurement of choline chloride using the above techniques requires solvents, expensive reagents, and trained operators, in addition to a considerable amount of time to successfully identify the amount of ChCl. The accuracy of these methods is also in question, as the Volhard method contains contaminants like NaCl and $CaCl_2$ that interfere with the measurement. Additionally, the Reinecke method is unsuitable for high concentrations of choline chloride (above 50%).

This application note demonstrates the advantages of using Fourier-transform near-infrared spectroscopy (FT-NIR) to quickly, accurately, and non-destructively analyze silage for choline chloride content. In addition, it is shown that FT-NIR can be used to acquire the concentration of choline chloride by scanning directly through the plastic bag that the ground cob arrives in, removing the need to unpack, soak, and titrate the samples.

Experiment

Twenty-five bags of silage were measured on the Antaris FT-NIR Analyzer at 4 cm^{-1} resolution using 8 co-averaged through-bag scans. The samples were then quantified using a silver chloride titration to assess the amount of choline chloride. These reference numbers were then combined with the spectral data in a calibration model using Thermo Scientific TQ Analyst™ Software, our chemometric analysis package.

The algorithm for the analysis was partial least squares, and the raw data was pretreated as a first derivative. Other pretreatments in this calibration include a multiplicative scatter correction and a Norris smooth with a segment length of 11 and a gap of 3.

Results

Spectral data collected by diffuse reflectance on silage is shown in Figure 1. There is a substantial amount of spectral variance between the samples, which is essential to establish a relationship between concentrations taken from reference values and FT-NIR data. Peaks in the near-IR spectrum that are due to varying concentrations of choline chloride will show differences in spectral absorbance.

Raw spectral data, however, is rarely used in FT-NIR spectroscopy. Mathematical processing of raw spectral data is common in near-IR spectroscopy due to NIR's broad, overlapping peaks. Using a first- or second-derivative treatment of the raw spectral data enhances the ability of chemometric models to pick out the spectral variation that is relevant to the component of interest. An example of first-derivative processed spectra is shown in Figure 2.

The calibration shows a strong correlation between the spectral information for choline chloride and the reference data from the Volhard titration. This is displayed as a calibration curve with the reference value for choline chloride on the x-axis and the value predicted by the near-IR calibration on the y-axis. If a calibration were theoretically perfect, then the concentrations predicted using the NIR data would match each reference value exactly. This would give a calibration curve with a perfectly straight line that had a slope of 1.0 and an intercept of 0.0. In reality, no calibration is quite this good, although the closer you can get to these parameters, the better the calibration. The calibration curve predicting choline chloride on silage results in a 0.98354 correlation coefficient, as shown in Figure 3. In addition, the calibration residual data (Figure 4), which shows the ± error of any calibration, describes a prediction accuracy of 1.6%. The regions that were used for analysis are shown in Figure 5.

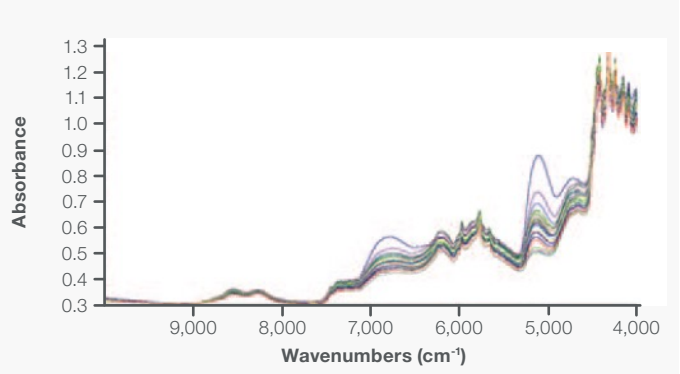

Figure 1. Spectra of choline chloride treated ground corncob.

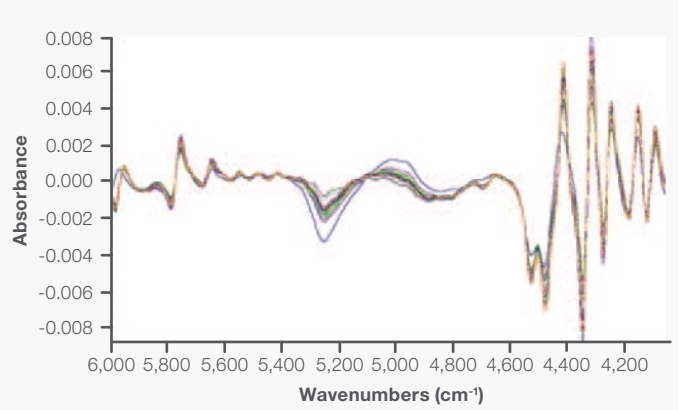

Figure 2. First-derivative processed spectra of choline chloride treated ground corncob.

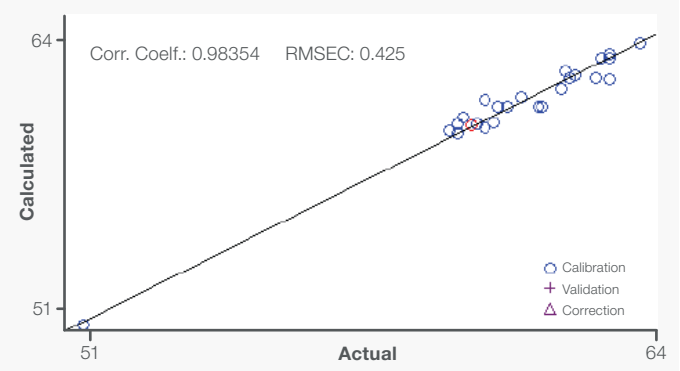

Figure 3. Calibration curve for choline chloride.

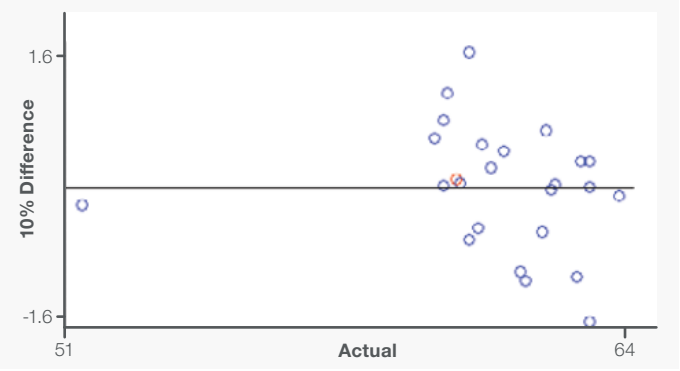

Figure 4. Residual for choline chloride.

Conclusion

In this application note, we determined that choline chloride concentration on ground corncob could be successfully quantified with a residual calibration error of ±1.6%. These analyses were performed in less than 6 seconds, while the reference data for each sample was generated over the course of 6 –12 hours due to the long extraction and titration times. This represents an enormous time savings over the traditional methods of analysis. In addition, the sample is analyzed non-destructively so it can still be used as feed, whereas other methods, like titration, destroy the entire sample. Analysis with the Antaris II FT-NIR Analyzer also allows the user to analyze for more than one component using the same spectrum. For example, if there was interest in the particle size of the cob granules or the concentration of other exogenous vitamins or chemicals in addition to choline chloride, the analysis time would be the same. Multivariate analysis techniques allow a single spectrum to be used for predicting the concentrations of multiple components in complex systems.

The data in this application note was collected using an older model Antaris FT-NIR Analyzer. Currently, Thermo Fisher Scientific offers an improved model, the Thermo Scientific Antaris II FT-NIR Analyzer, which offers superior speed and performance over its predecessor model.

Index	Measurement Location / Range	CC
1	6134.45 - 6090.10	+
2	5974.39 - 5937.75	+
3	5708.26 - 5660.05	+
4	8531.54 - 8221.05	+

Figure 5. Region selection window in TQ Analyst Software.

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