Application Note AN-NIR-093

Quality Control of fermentation processes

Multiparameter determination within one minute

The production of biofuels from renewable feedstock has grown immensely in the past several years. Bioethanol is one of the most interesting alternatives for fossil fuels, since it can be produced from raw materials rich in sugars and starch. Ethanol fermentation is one of the oldest and most important fermentation processes used in the biotechnology industry. Although the process is well-known, there is a great potential for its improvement and a proportional reduction in production costs. Due to the seasonal variation of feedstock quality, ethanol producers to need to monitor the fermentation process to ensure the same quality product is achieved.

Near-infrared spectroscopy (NIRS) offers **rapid and reliable prediction** of ethanol content, sugars, Brix, lactic acid, pH, and total solids at any stage of the fermentation process.

EXPERIMENTAL EQUIPMENT

Production of ethanol from corn goes through three typical steps: milling / liquefaction of corn into starch mash, fermentation of starch mash with yeast, and finally purification of the resulting ethanol by distillation. A total of 206 samples (117 for Brix index) of fermentation mash were analyzed on the DS2500 Solid Analyzer. Due to the large amount of solids present in the samples, all measurements were performed in reflection mode using the DS2500 Large sample cup (Figure 1). The samples were measured in rotation to collect spectral data from several areas. Spectral averaging of signals from several spots helped to reduce sample inhomogeneity. The Metrohm software package Vision Air Complete was used for all data acquisition and prediction model development.

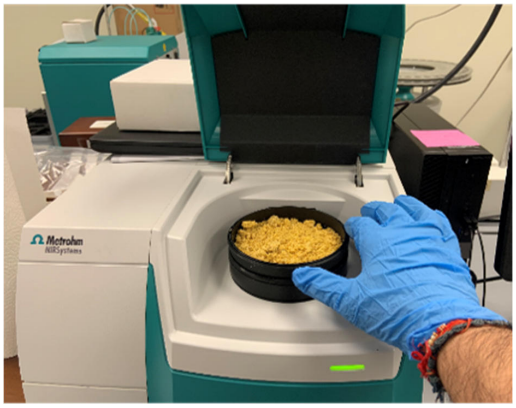

Figure 1. Fermentation mash sample placed on the DS2500 Solid Analyzer.

Table 1. Hardware and software equipment overview

Equipment	Metrohm number
DS2500 Analyzer	2.922.0010
DS2500 Large Sample Cup	6.7402.050
Vision Air 2.0 Complete	6.6072.208

RESULT

All 206 measured Vis-NIR spectra (Figure 2) were used to create a prediction model for quantification of the key fermentation parameters. The quality of the prediction model was evaluated using correlation diagrams, which display a very high correlation between the Vis-NIR prediction and the reference values. The respective figures of merit (FOM) display the expected precision of a prediction during routine analysis.

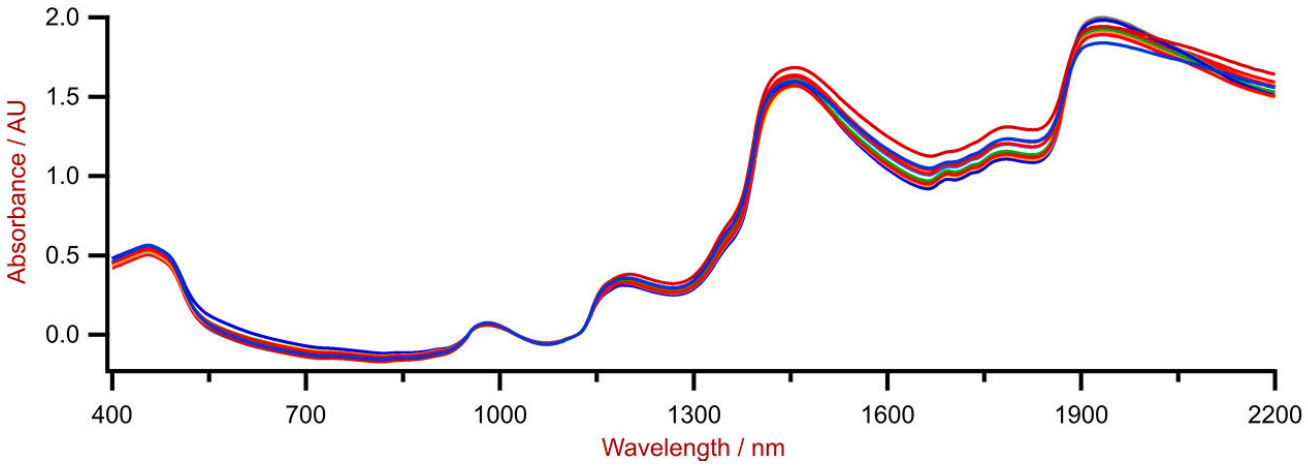

Figure 2. Vis-NIR spectra of fermentation mash samples analyzed on a DS2500 Solid Analyzer.

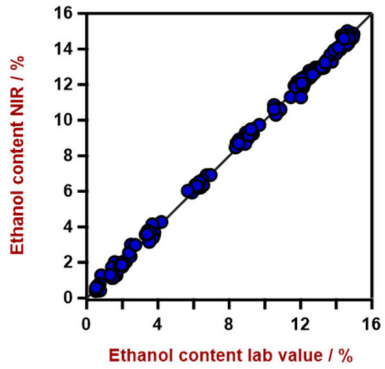

Figure 3. Correlation diagram for the prediction of ethanol content using a DS2500 Solid Analyzer. The ethanol content lab value was evaluated using HPLC.

Table 2. Figures of merit for the prediction of ethanol content using a DS2500 Solid Analyzer.

Figures of merit	Value
R_2	0.998
Standard error of calibration	0.21%
Standard error of cross-validation	0.22%

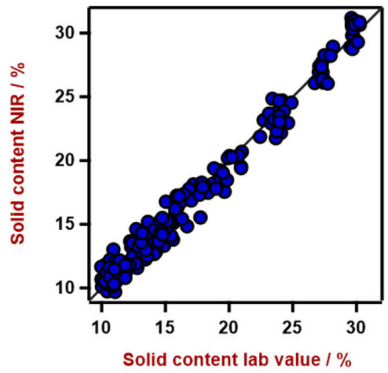

Figure 4. Correlation diagram for the prediction solid content using a DS2500 Solid Analyzer. The lab value was evaluated by LOD balance.

Table 3. Figures of merit for the prediction solid content using a DS2500 Solid Analyzer.

Figures of merit	Value
R_2	0.982
Standard error of calibration	0.87%
Standard error of cross-validation	1.06%

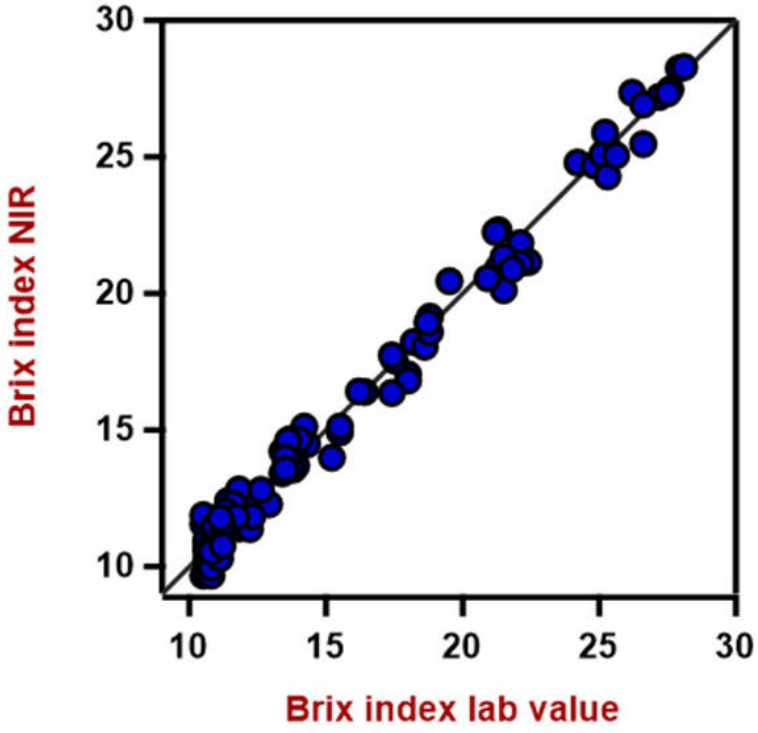

Figure 5. Correlation diagram for the prediction of Brix index values. The lab value was measured using a refractometer.

Table 4. Figures of merit for the prediction of Brix index values.

Figures of merit	Value
R_2	0.987
Standard error of calibration	0.66
Standard error of cross-validation	0.87

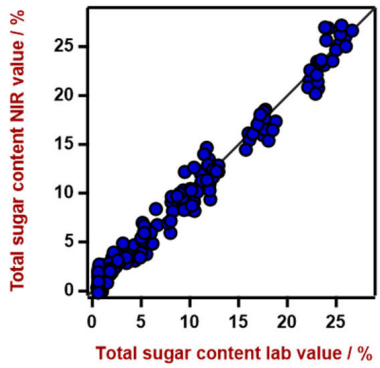

Figure 6. Correlation diagram for the prediction of the total sugar content. The total sugar content lab value was measured using HPLC.

Table 5. Figures of merit for the prediction of the total sugar content.

Figures of merit	Value
R_2	0.981
Standard error of calibration	1.09%
Standard error of cross-validation	1.30%

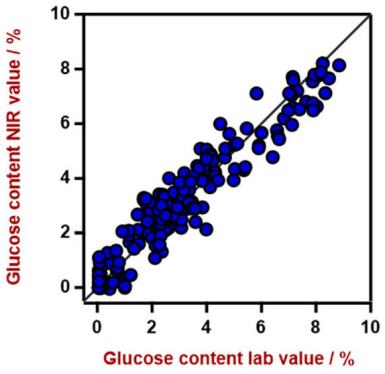

Figure 7. Correlation diagram for the prediction of glucose content. The glucose content lab value was measured using HPLC.

Table 6. Figures of merit for the prediction of the glucose content.

Figures of merit	Value
R_2	0.920
Standard error of calibration	0.70%
Standard error of cross-validation	0.86%

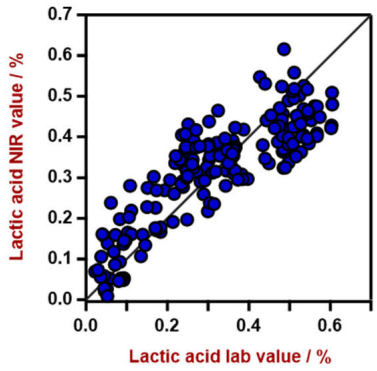

Figure 8. Correlation diagram for the prediction of lactic acid content. The lactic acid lab value was evaluated using HPLC.

Table 7. Figures of merit for the prediction of lactic acid content.

Figures of merit	Value
R_2	0.722
Standard error of calibration	0.09%
Standard error of cross-validation	0.10%

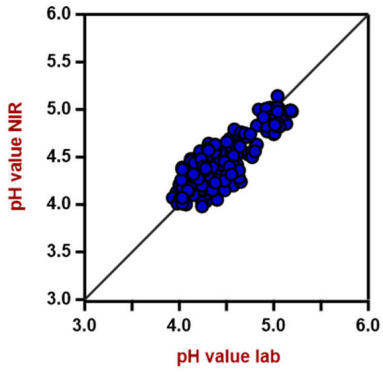

Figure 9. Correlation diagram for the prediction of pH value. The pH lab value was measured using a pH meter.

Table 8. Figures of merit for the prediction of pH value.

Figures of merit	Value
R_2	0.734
Standard error of calibration	0.17
Standard error of cross-validation	0.19

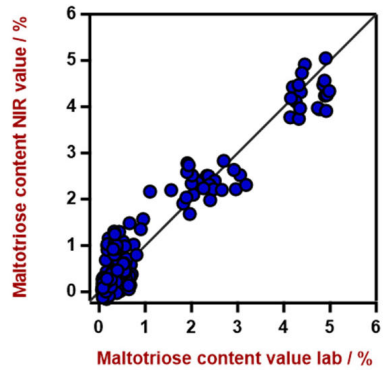

Figure 10. Correlation diagram for the prediction of maltotriose content. The maltotriose lab value was measured using HPLC.

Table 9. Figures of merit for the prediction of maltotriose content.

Figures of merit	Value
R_2	0.928
Standard error of calibration	0.36%
Standard error of cross-validation	0.42%

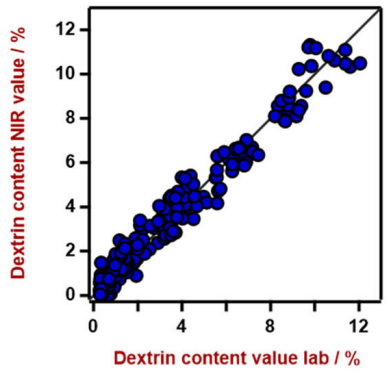

Figure 11. Correlation diagram for the prediction of dextrin content. The dextrin lab value was measured using HPLC.

Table 10. Figures of merit for the prediction of dextrin content.

Figures of merit	Value
R_2	0.964
Standard error of calibration	0.60%
Standard error of cross-validation	0.68%

CONCLUSION

This application note demonstrates the feasibility to determine multiple key parameters of the fermentation process with NIR spectroscopy. Corn fermentation is a well-established process which typically runs for 55–60 hours. Samples are extracted from fermenters every few hours and sent to the laboratory for analytical measurement. Several analytical methods need to be used to monitor key quality parameters for the fermentation process. Vis-NIR spectroscopy enables a fast alternative with high accuracy, and therefore represents a suitable single method to monitor the fermentation process.

Table 11. Time to result overview for the different parameters

Parameter	Method	Time to result
Ethanol, sugars	HPLC	30–45 min
Brix index	Refractometer	3–5 min
pH	pH meter	3–5 min
Solids	LOD Balance	10–15 min

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DS2500 Solid Analyzer

Spectroscopie proche infrarouge robuste pour le controle de la qualité en laboratoire et en environnement de production.

L'analyseur DS2500 Analyzer est la solution éprouvée et souple destinée aux analyses de routine de matières solides, de crèmes et, en option, de liquides, tout au long de la chaine de fabrication. Sa conception robuste fait du DS2500 Analyzer un appareil insensible à la poussière, à l'humidité, aux vibrations ainsi qu'aux variations de température, et donc particulièrement adapté aux rudes conditions d'un environnement de production.

Le DS2500 couvre l'ensemble de la gamme spectrale de 400 à 2 500 nm et fournit des résultats exacts et reproductibles en moins d'une minute. Le DS2500 Analyzer répond aux exigences de l'industrie pharmaceutique et représente une aide précieuse pour les opérations de routine quotidiennes grace à sa simplicité d'utilisation.

Grace à des accessoires parfaitement adaptés à l'appareil, il atteint des performances sans précédent avec tous les types d'échantillons, quel que soit le défi qu'ils opposent (matières solides à gros grains comme les granulats ou échantillons semi-solides ou liquides telles les crèmes). La productivité lors de mesures de matières solides peut encore etre augmentée par l'utilisation du MultiSample Cup, lequel permet des mesures automatisées en série jusqu'à un maximum de 9 échantillons.

DS2500 Récipient d'échantillon, grand

Grand récipient d'échantillon pour l'enregistrement de spectre par réflexion de poudres et de granulés en différents points de l'échantillon avec le NIRS DS2500 Analyzer.

Vision Air 2.0 Complete

Vision Air - logiciel universel de spectroscopie.

Vision Air Complete est une solution logicielle moderne et simple d'utilisation pour une application dans un environnement réglementé.

Apercu des avantages de Vision Air :

- Des applications logicielles individuelles avec interface utilisateur adaptée sont le garant d'un maniement intuitif et simple
- Établissement et suivi simples des procédures de travail
- Base de données SQL pour une gestion sure et simple des données

La version Vision Air Complete (66072208) comprend toutes les applications d'assurance qualité par spectroscopie Vis-NIR :

- Application de gestion des instruments et des données
- Application de développement de méthodes
- Application d'analyse de routine

Autres solutions Vision Air Complete :

- 66072207 (Vision Air Network Complete)
- 66072209 (Vision Air Pharma Complete)
- 66072210 (Vision Air Pharma Network Complete)