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.

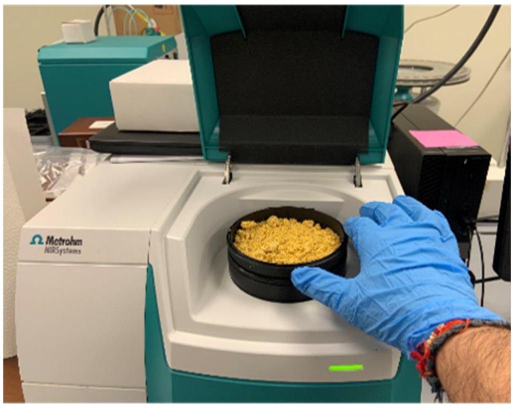

Figure 1. Fermentation mash sample placed on the DS2500 Solid Analyzer.

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.

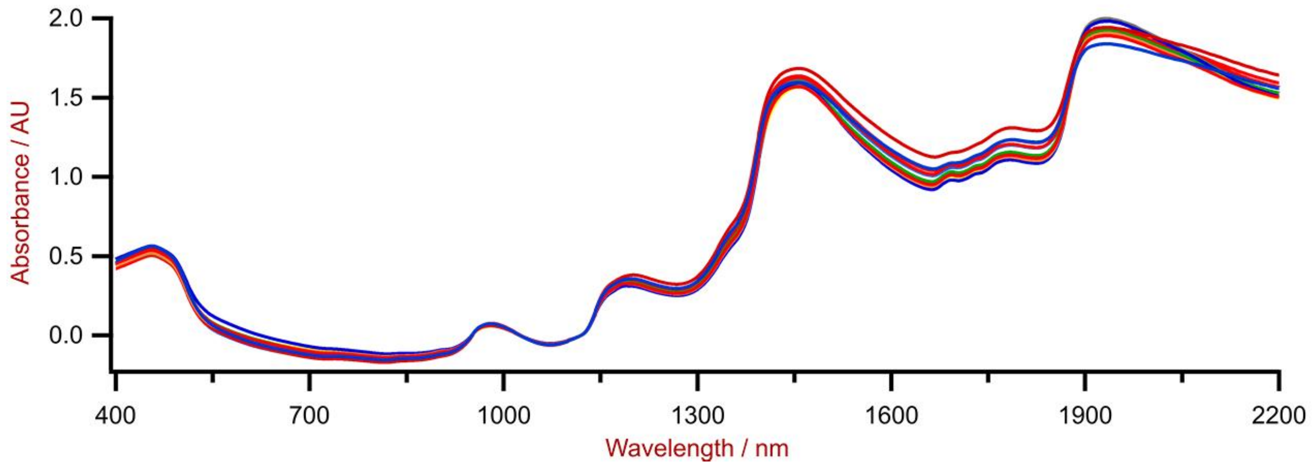

Figure 2. Vis-NIR spectra of fermentation mash samples analyzed on a DS2500 Solid Analyzer.

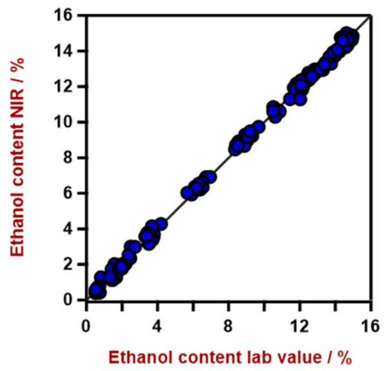

Figure 3. Correlation diagram for the prediction of ethanol content using a DS2500 Solid Analyzer. The ethanol content lab value was evaluated using HPLC.

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%

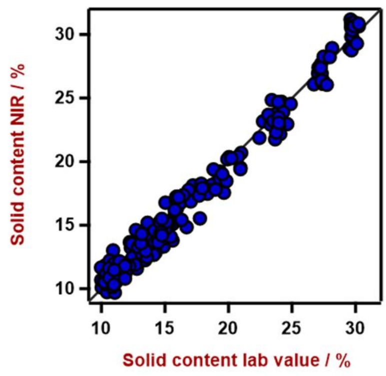

Figure 4. Correlation diagram for the prediction solid content using a DS2500 Solid Analyzer. The lab value was evaluated by LOD balance.

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%

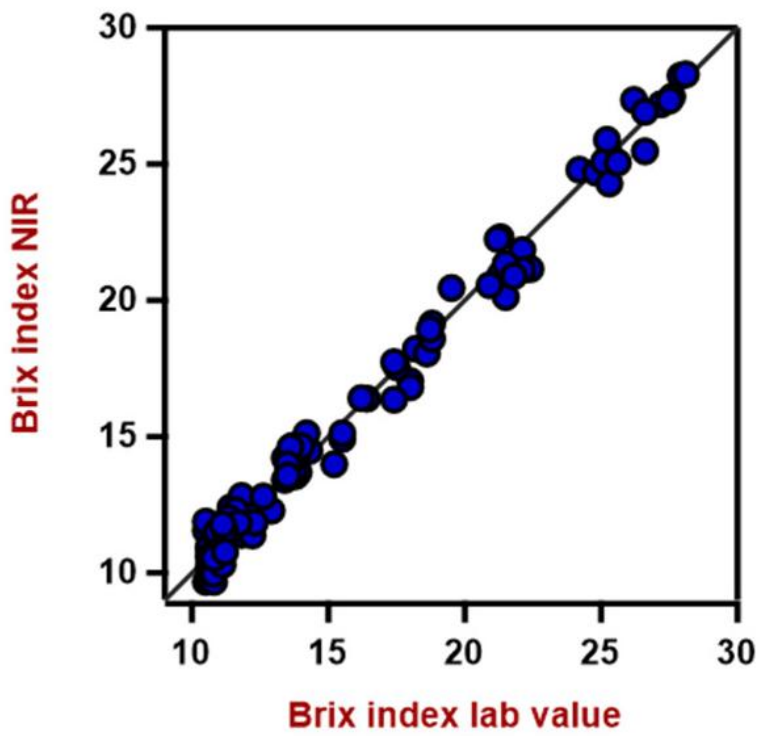

Figure 5. Correlation diagram for the prediction of Brix index values. The lab value was measured using a refractometer.

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

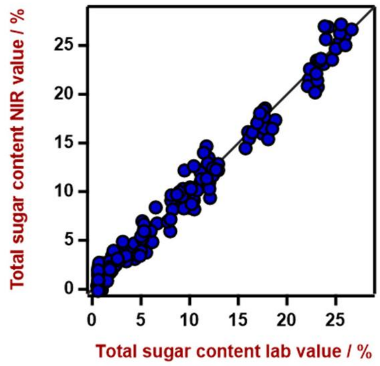

Figure 6. Correlation diagram for the prediction of the total sugar content. The total sugar content lab value was measured using HPLC.

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%

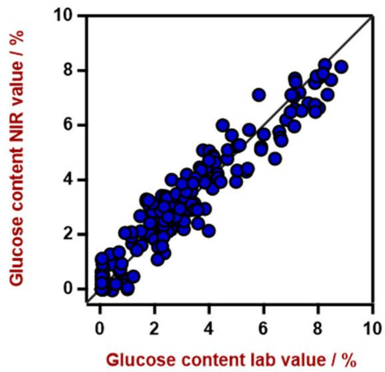

Figure 7. Correlation diagram for the prediction of glucose content. The glucose content lab value was measured using HPLC.

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%

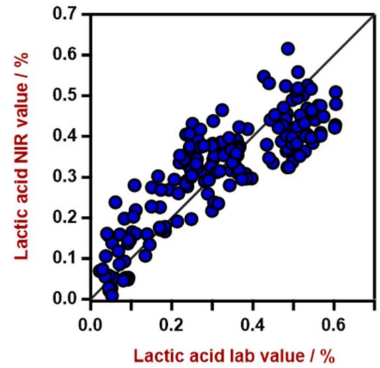

Figure 8. Correlation diagram for the prediction of lactic acid content. The lactic acid lab value was evaluated using HPLC.

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%

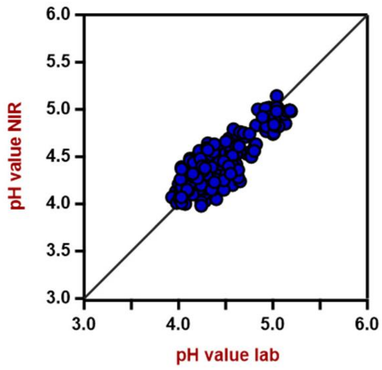

Figure 9. Correlation diagram for the prediction of pH value. The pH lab value was measured using a pH meter.

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

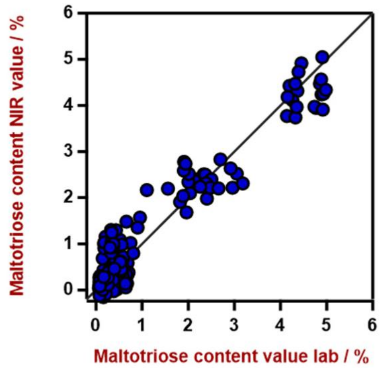

Figure 10. Correlation diagram for the prediction of maltotriose content. The maltotriose lab value was measured using HPLC.

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%

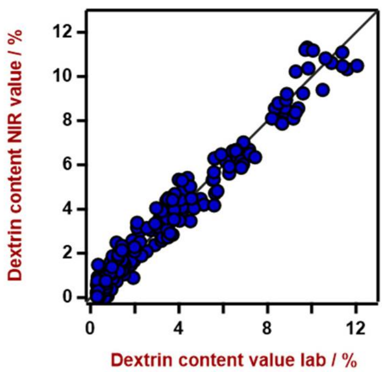

Figure 11. Correlation diagram for the prediction of dextrin content. The dextrin lab value was measured using HPLC.

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

CONTACT

メトロームジャパン株式会社
143-0006 東京都大田区平和島6-1-1
null 東京流通センター アネックス9階

metrohm.jp@metrohm.jp

DS2500 Solid Analyzer

ラホおよひ生産環境における品質管理用の堅牢な近赤外分光法。

DS2500 Analyzerは、生産チェーン全体に沿った固形物、クリーム、およひオフションとしての液体のルーチン分析に実績のあるフレキシフルなソリューションてす。頑丈な仕様により、DS2500 Analyzerは粉塵、湿気、振動や温度変動に強い為、過酷な生産環境ての使用に理想的てす。

DS2500は400 ~ 2500 nmのスヘクトル範囲全体をカハーし、1分以内に正確て再現性の高い結果を提供します。DS2500 Analyzerは製薬業界の要件を満たしており、簡単な操作により日常的な作業においてユーサーをサホートします。

装置に完全に適応した付属品により、 顆粒のような粒の荒い固形物、またはクリームのような半固形液体サンフルなとのあらゆる困難なタイフのサンフルにおいても、最良の結果を得ることかてきます。固形物の測定においては、9つまてのサンフルのシリースの自動測定を可能にするMultiSample Cupを使用することて、生産性を高めることかてきます。

DS2500

NIRS DS2500 Analyzerを用いた、様々なサンフル位置における反射中の粉末および顆粒のスヘクトル記録のための、大きなサンフル容器てす。

Vision Air 2.0 Complete

Vision Air - 汎用性に優れた分光法ソフトウェア。

Vision Air Complete は、規制環境下ての使用のための、操作の容易な最新のソフトウェアソリューションてす。

Vision Air の利点の概要:

- 調整済みのユーザーインターフェースを伴う個別のソフトウェアアフリケーションにより、直観的かつ容易な操作か保証されます。
- 作業手順の容易な作成およひメンテナンス
- 安全かつ容易なテータ管理のための SQL テータヘース

ハーション Vision Air Complete (66072208) には、可視近赤外分光法を用いた品質管理のための全てのアフリケーションか含まれています:

- 装置管理およひテータ管理のためのアフリケーション
- メソット開発のためのアフリケーション
- ルーチン分析のためのアフリケーション

その他の Vision Air Complete ソリューション:

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