Application Note AN B408

Determination of nitrogen compounds in beer using MIR spectroscopy

The analysis of nitrogen compounds in beer yields important information for quality control. Mid-infrared spectroscopy using microplates in conjunction with a chemometric evaluation method allows an easy and simultaneous quantification of total dissolved nitrogen, free amino compounds and high-molecular weight nitrogen compounds. The high sample throughput and the lack of additional chemicals are further advantages of this new method.

Nitrogen compounds in the brewing process

Nitrogen compounds, especially proteins, peptides and free amino acids play an important role in the brewing process; [1]. Amino acids and ammonium salts, for example, are essential for the fermentation process as they are the main nitrogen source for the yeast. Also, their concentrations are closely linked with the production of fermentation by-products. The filterability of the beer strongly depends on the concentration of high-molecular weight protein. Medium and especially high molecular weight nitrogen compounds directly influence the foam stability, the taste of the beer and colloidal stability (storage quality). As the different nitrogen compounds have different effects on these product features, it is not only necessary to quantify the total amount of nitrogen, but also the concentrations of the individual substance groups [2] Methods to determine the nitrogen compounds in the initial, intermediate and final products of the brewing process are defined by the respective brewing associations. All these methods are labour intensive, time consuming and require chemicals that are partly harmful or toxic. Moreover, these methods do not allow an analysis that can be performed at-line, during the brewing process.

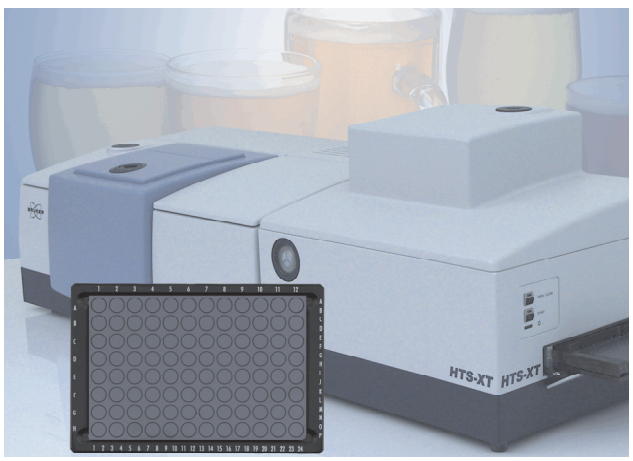

Fig 1.) Silicon microplates for IR spectroscoy; IR microplate module HTS-XT

MIR Spectroscopy and chemometric evaluation

For MIR spectra both the qualitative composition of a sample and the concentration of individual components can be determined. Classic evaluation methods assess the MIR spectra on the basis of the Lambert-Beer law by using the height or the area of a band that is specific for a certain component. For the analysis of IR spectra resulting from complex mixtures of many substances, this method often is not applicable due to overlapping bands. Therefore chemometric evaluation methods like PLS have to be applied.

For calibration the concentration values of representative calibration samples have to be determined as exact as possible, using an independent reference method. Then the Partial Least Squares (PLS) algorithm calculates a mathematical model for the relation between the calibration spectra and the reference values. After setting up, optimizing and validating the model, the concentrations of unknown samples can be determined quickly and easily on the basis of their spectra. The achieved accuracy of the results, depends on the accuracy of the reference method (i.e. the results can not be better than the reference method!).

Many substances absorb strongly in the MIR region so that, according to the Lambert-Beer law, only a small pathway of the sample is required. As the OH-bands of water broadly overlap with the bands of other sample components the MIR-analysis of aqueous samples is only possible in extremely thin cuvettes (< 10 µm) or after removal of the water. On the microplate (Fig. 1) this can be accomplished by drying a few microlitres of the sample on a sample position. The result is a thin film of the dissolved constituents that can now be measured in transmittance using the MIR spectroscopy. Another advantage of this measurement method is the high sample throughput with typically 96 or up to 384 individual measurements per microplate.

Samples and Reference Method

Our sample set comprised 31 different beers purchased from local vendors. Besides 27 Pilsener beers, the sample set also included one non-alcoholic beer and three black beers for test purposes. Another 6 „artificial" beer samples were produced by ultrafiltrating one beer (Vivacell 70, MWCO=5000; Vivascience, Hannover) and preparing different mixtures of the original, the retentate and the filtrate.

According to MEBAK (Mitteleuropäische Brautechnische Analyse-Kommission), all samples were analyzed for free amino nitrogen (FAN) using the photometric ninhydrin method and for the total nitrogen (TN) and for high molecular weight nitrogen (HMN) (nitrogen precipitating after magnesium sulphate treatment) using the Kjeldahl method. The determinations were repeated two times and during the ninhydrin test, they were repeated three times. The analysis results have been in the range of 63 - 194 mg/l

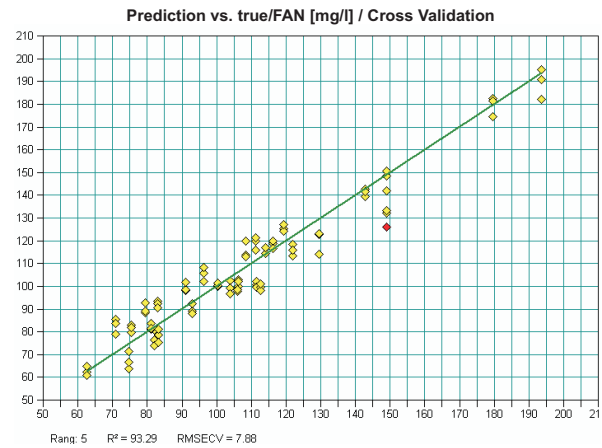

Fig 2.) Result of a cross validation for the determination of FAN in beer ensuring defined drying conditions.

FAN, 461 - 1434 mg/l (TN) and 83 - 185 mg/l (HMN). To check the reproducibility of the reference measurement, one beer sample were analyzed five times revealing a relative standard deviation of 2.5 % (FAN), 3.0 % (TN) and 1.0 % (HMN).

The MIR measurements were performed using Bruker Optic's HTS-XT microtiterplate reader coupled to TENSOR 27 FT-IR spectrometer. From each sample, 10 µl were pipetted on three sample position on silicon microtiterplate and incubated to dry at room temperature (ca. 15 - 20 min). The measurement data was evaluated chemometrically using a PLS algorithm (Partial Least Squares) of the OPUS/QUANT software.

Results

Because of the small number of samples, the "Cross Validation" option was chosen for the verifying the PLS-calibration. In this case, one sample is excluded from the sample set before the calibration started. The remaining samples are used to calibrate the system. Then, the excluded sample (that is considered as an unknown sample) is used to test the chemometric model. This procedure is repeated for each sample of the sample set. In figure 2, the results of the cross validation for FAN are plotted against the corresponding reference values. Table 1 includes the respective statistical parameters for all three measured components. The mean error of prediction is between 5 - 7 %, referring to the mean value of the reference data.

Conclusions

Using MIR spectroscopy and chemometric evaluation methods, the concentration of the different nitrogen compounds in beer can be determined fast and accurately. A major

advantage of this analytical method is the fact that the concentration of several components like total nitrogen, free amino nitrogen and high molecular weight nitrogen can be determined simultaneously with only one measurement and without requiring additional chemical reagents. A further improvement of this method can be achieved, by including more samples in the calibration set and ensuring defined drying conditions.

Analytical Parameter	Concentration (mg/l)	R^2	RMSECV (mg/l)
Free Amino Nitrogen	63-194	0,93	7,9
High Molecular Weigth Nitrogen	83-285	0,91	11,4
Total Nitrogen	623-1434	0,94	44,0

Table 1: Results of the IR-spectroscopic determination of nitrogen compounds in 31 beers.

References

[1] Narziß, L.: Abriß der Bierbrauerei, Enke, Stuttgart, 1995

[2] Schlecht, E.: Brauwelt 126, 54-60 (1986)

[3] Pfenninger, H. (Hrsg.): Brautechnische Analysen methoden (Band II), Selbst-verlag der MEBAK, Weihenstephan, 1993

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