

New spectroscopic insights for the identification of mycotoxins in cereals

Claudia Rasch*, Michael U. Kumke, Hans-Gerd Löhmannsröben

University of Potsdam, Department of Chemistry (Physical Chemistry)
Karl-Liebknecht-Str. 24-25, 14476 Potsdam-Golm (Germany)
*E-Mail: Claudia.Rasch@uni-potsdam.de

Introduction

Mycotoxins are toxic fungal metabolites that may contaminate primary food products such as cereals, nuts and fruits. The most predominant mycotoxins in Europe among others are the *Aflatoxins* and *Ochratoxins* produced by storage fungus, such as *Aspergillus* and *Penicillium* species, and mycotoxins formed by field-borne *Fusarium* species, for example *Zearalenone* and *Deoxynivalenol*.^[1]

Because of the potential health hazards for humans, the monitoring of food and feed for the presence of these toxins are highly important with respect to the food safety aspects. Therefore, an urgent need for reliable, low-cost and easy-to-use experimental setups exists. Legislation guidelines regarding the allowed levels of mycotoxins in food and feed products as well as in raw materials are presented by the FAO, updated in 2003.^[2] A reliable and sensitive in-situ detection of fungi contamination and mycotoxins in the raw materials at the beginning of the food production chain is indispensable in order to increase food and feed safety to the standards required.

The use of spectroscopic methods in food control and food monitoring is increasing rapidly, especially in combination with chemometric tools (e.g. Partial Least Square, Principal Component Analysis, Multivariate Curve Resolution).^[3]

Non-destructive methods, such as absorption, fluorescence and reflection spectroscopy, are powerful methods for the detection of mycotoxins in solution and on the surface of grains and flour. Because of its outstanding sensitivity fluorescence spectroscopy based techniques are especially suited for the in-situ detection of mycotoxins such as *Ochratoxins*, *Aflatoxins* as well as *Zearalenone*. These mycotoxins can be monitored with an excitation in the spectral range of $200 \text{ nm} < \lambda_{\text{ex}} < 400 \text{ nm}$ and a detection wavelength $\lambda_{\text{em}} > 420 \text{ nm}$. In combination with absorption and reflection measurements, qualitative and quantitative information on the mycotoxins present can be obtained. In addition, near infrared diffuse reflection spectra can yield further information on ingredients, moisture content, and presence (or absence) of fungi in the sample.^[4]

Experimental setup

Near infrared radiation covers by definition the wavelength range from 780 to 2500 nm. When radiation interacts with a sample, the incident radiation may be absorbed, transmitted or reflected. The relative contribution of each process depends on the chemical composition and the physical parameters of the sample. In case the rough surfaces of the grains reflect diffusely without penetration into the sample like regular reflectance, no light attenuation takes place. Quantitative NIR analysis requires instruments which are designed for reflectance analysis, e.g. the integrating sphere (figure 1). The obtained data can be analysed by the Kubelka-Munk-theory.^[5]

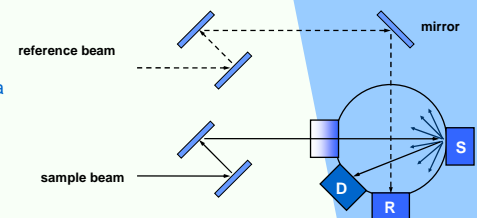


Figure 1: schematic setup of an integrating sphere (S – sample, R- reference, D – detector)

Results and Chemometrics

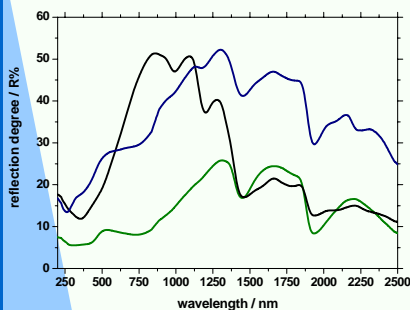


Figure 2: Reflection spectra of uncontaminated wheat (black), wheat with fungal contamination (blue), pure culture *Penicillium verrucosum* (green)

In figure 2 three typical diffuse reflectance spectra in the spectral range between 200 and 2500nm of uncontaminated wheat, kernels with fungal contamination and the pure culture of *Penicillium verrucosum* are shown. The infested kernels differ in size and moisture content. The form and intensity distribution of the spectral bands illustrates the change in the spectral region below 1200nm and an increase in reflection above 1200nm.

It is also possible to qualitatively and quantitatively account for the ingredients. On the right side (figure 3) five spectra of wheat kernels with different moisture, starch and protein content are shown. The spectra are clearly very similar and are dominated by the water spectrum with its characteristic overtone bands (OH-bands at 970, 1440 nm) and a combination band at 1940 nm.

The application of diffuse reflectance spectroscopy on grains is complicated by the large variance in their size, shape, colour, density, composition (water, starch, protein) and texture (figure 4). Due to the similarity of the spectra sophisticated multivariate statistical techniques are indispensable to extract further information (e.g., on contaminations) from the NIR spectrum.

The raw NIR data are pre-treated as shown in figure 5. The plotted NIR spectra are transformed into the common logarithm of R ($R=R\%/100\%$). Abnormal spectra are not be sorted out. A baseline correction or smoothing of the spectra is the next step, but it can cause a loss of valuable information. After transformation to the first or second derivative, the SNV transformation or MSC leads finally to the data used in the multivariate analysis (PCA, PLS, MCR).

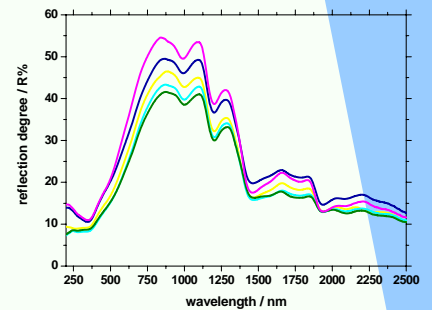


Figure 3: Reflection spectra of uncontaminated wheat with variable moisture content: 11% (green), 15% (light blue), 18% (yellow), 21% (blue) and 25% (pink)

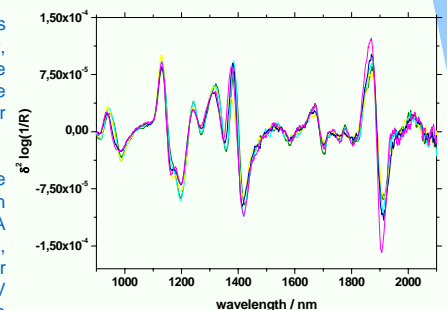


Figure 5: Second derivatives of the reflection spectra of uncontaminated wheat with variable moisture content: 11% (green), 15% (light blue), 18% (yellow), 21% (blue) and 25% (pink)



Figure 4: Great variance of wheat

Conclusion

With diffuse reflection spectroscopy quantitative and qualitative information on the ingredients, moisture content and the presence (or absence) of fungi in the sample can be obtained. Further, the experimental NIR spectra depend on the shape, colour, density and texture as well as moisture content and composition of the kernels. But with chemometric tools it is expected to eliminate these disturbing factors and reach out specifically for the chemical information of the sample. On the basis of PLS composition data can be obtained and with PCA respectively MCR the presence (or absence) of fungi will be demonstrated.

References

- [1] EMAN - European Mycotoxin Awareness Network (www.mycotoxins.org)
- [2] FAO. Worldwide regulations for mycotoxins in food and feed in 2003; *FAO Food and Nutrition Paper 81, 2004*
- [3] Kessler, Waltraud (ed.); *Multivariate Datenanalyse für die Pharma-, Bio- und Prozessanalytik*, Wiley VCH, Weinheim 2007
- [4] Williams, Phil; *Near Infrared Spectroscopy in Food Science and Technology* (edited by Y. Ozaki, W.F. McClure, A.A. Christy), Jon Wiley & Sons, Hoboken (New Jersey) 2006
- [5] Kessler, Rudolf W. (ed.); *Prozessanalytik. Strategien und Fallbeispiele aus der industriellen Praxis*, Wiley VCH, Weinheim 2006

Acknowledgement

This study is financially supported in the joint research project „ProSenso.net2“ (PSn2) which is funded by the German Federal Ministry of Education and Research (BMBF).