

Spectroscopic identification of mycotoxins in cereals

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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 fungi, 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 mycotoxins is of utmost importance. 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 and Principal Component Analysis).^[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. 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 that have been designed for reflectance analysis, e.g. the integrating sphere (figure 1). 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, respectively. The obtained data can be analysed by the Kubelka-Munk-theory.^[5]

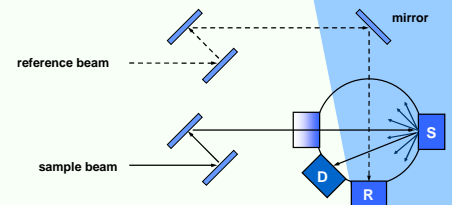


Figure 1: schematical setup of an integrating sphere (S – sample, R- reference, D – detector)

Results

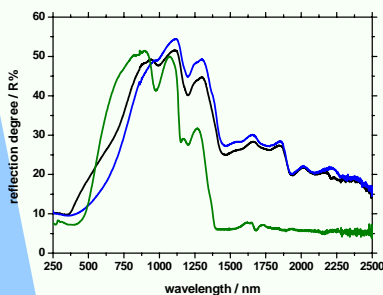


Figure 2: Reflection spectra of uncontaminated rye (black) and with fungal decay (blue) as well as *Fusarium poae* (green), against 99% Spectralon

In figure 2 three typical diffuse reflectance spectra in the spectral range between 250 and 2500nm of uncontaminated rye, kernels with fungal contamination and the pure culture of *Fusarium poae* are shown. The infested kernels differ in colour and moisture content. The form and intensity distribution of the spectral bands illustrates the colour change in the spectral region below 1000nm and an increase in reflection above 1000nm.

It is also possible to qualitatively and quantitatively account for the ingredients. On the right side (figure 3) five spectra of rye 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. From the second derivative spectra the moisture content can be determined. But 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.

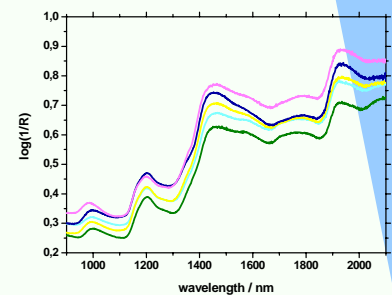


Figure 3: Reflection spectra of uncontaminated rye with variable moisture content: 11% (green), 15% (light blue), 17% (yellow), 22% (blue) and 28% (pink)

Chemometrics

The raw NIR data are pre-treated as shown in figure 4. The plotted NIR spectra are transformed into the common logarithm of R ($R=R\%/100\%$). Abnormal spectra will 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 and the MSC leads finally to the data used in the multivariate analysis (PCA, PCR, PLS, MLR).

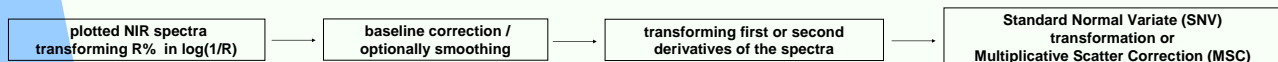


Figure 4: Pretreatment of NIR data before data analysis

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 the presence (or absence) of fungi will be demonstrated.

References

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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).