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Inline Determination of Phytic Acid Content in Cereals During Enzymatic Phosphorus-Adjustment

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PURPOSE OF THE ABSTRACT

Phosphorus (P) is essential for the development and growth of plants and livestock. In seeds and grain phytic acid (InsP6) is the main source and storage form of P. However, monogastric species cannot digest InsP6 present in plant-based feeds due to limited activity of endogenous intestinal InsP6-hydrolyzing enzymes. Therefore, the manure contains high amounts of residual P. The subsequent manure application not only impacts agricultural productivity but also poses threats to water quality (eutrophication), resulting in declined biodiversity. Moreover, InsP6 is considered as an important antinutrient in animal nutrition, since it binds essential mineral cations. To increase InsP6 digestibility, phytases are often supplemented to animal feed rations. However, about 50% of InsP6 continuous to be excreted and is therefore used inefficiently by the animals.

The aim is to establish an enzymatic conditioning process to adjust the P content in cereal based feeds as well as to recover excess P. Rye bran, a by-product of the milling industry that is widely used in compound feeds, is used as model compound. In a One-Pot system, InsP6 is solubilized in water via mass transfer and subsequently hydrolyzed by the activation of intrinsic enzymes. In the liquid phase Fourier-Transform-Infrared-Spectroscopy (FT-IR) is used for concentration profiling. FT-IR allows fast response to changing process conditions, and it is a safe, precise and reliable inline analysis. However, the aqueous system poses a challenge, since large parts of the spectra are overlaid by OH-bands. Furthermore, the absorption bands of InsP6 and the hydrolytic product (H3PO4) are identical and, therefore, cannot be distinguished. Nevertheless, by applying a mass balance for P and assuming that all P-O absorption in the liquid phase belongs to either InsP6 or H3PO4, we were able to develop a chemometric model for the indirect determination of InsP6 in the biological material during the conditioning.
The experiments were carried out based on previous studies for the activation of intrinsic InsP6-hydrolyzing enzymes in rye bran. For model calibration KH2PO4 at 18 different concentrations ranging von 0-250 mM were used in duplicates as internal standard and for data pretreatment a baseline correction and a maximum normalization were executed. The presence of the counter ion showed no interfering effect, however, the spectra experienced pH-dependency. Therefore, the pH of the calibration solutions was set to pH 6.5, since it is the natural occurring pH when incubating rye bran in water. For data evaluation, the conditioning experiments were stopped at certain time points (1 h, 2 h, 4 h) and the remaining InsP6 was determined by offline analytics (HPLC). The results were compared to the chemometric data and showed an RMSE of 13.1 mgP·100gbran-1 equaling 2.2 mgInsP6·100gbran-1. Thus, the data derived from chemometrics is in agreement with those obtained by offline analysis with a high accuracy. Based on the real-time analysis developed, we were able to show that mass transfer is the rate-limiting step in this experimental setup.

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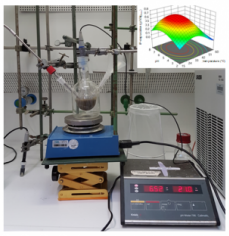
FIGURES



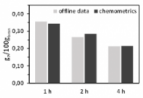


FIGURE 1
Experimental setup for P-adjustment in rye bran and the development of FT-IR based inline analysis

FIGURE 2
Comparison of the chemometric data with those obtained by offline analysis

KEYWORDS
phytate | biocatalysis | inline analytic | FT-IR

BIBLIOGRAPHY

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