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THE DEVELOPMENT AND VALIDATION OF THE CALIBRATION MODEL FOR THE VIS-NIR SPECTROMETER USED FOR THE EVALUATION OF DEOXYNIVALENOL CONTENT IN WHEAT GRAIN DIRECTLY DURING COMBINE HARVEST

Summary

The study is an attempt to build the calibration model for the VIS-NIR spectrometer used for measuring deoxynivalenol (DON) concentration in winter wheat grain at the stage of combine harvesting. In order to achieve this goal during the harvest season of 2011 the research material of 14.5 thousand radiation absorption spectrums and 283 grain samples was collected in field conditions. The low concentration of deoxynivalenol in natural grain samples did not allow a construction of the calibration model. Therefore, the PLS models (R^2≈0.9; RMSECV≈3–4 mg·kg^{-1}) were developed on the basis of 60 samples prepared in laboratory conditions. The model set, which consisted of samples with the DON concentration of 0-32 mg·kg^{-1}, was additionally supplemented with the results obtained for 58 samples from organic farming. The models were validated on the basis of the spectrums recorded in field conditions. The accuracy of prediction of the DON concentration by means of the model constructed on the basis of both sample collections was higher, but it was still insufficient.
The study was a part of the development project No. R12 0073 06 entitled "Development and validation of the technology for separation grain stream during cereals selective harvesting ", financed by the Polish Ministry of Science and Higher Education.
***Key words**: food safety, deoxynivalenol, VIS-NIR spectrometry, PLS calibration model*

OPRACOWANIE I WALIDACJA MODELU KALIBRACYJNEGO DLA SPEKTROMETRU VIS-NIR PRZEZNACZONEGO DO OCENY ZAWARTOŚCI DEOKSYNIWALENOLU W ZIARNIE PSZENICY BEZPOŚREDNIO PODCZAS KOMBAJNOWEGO ZBIORU

Streszczenie

W pracy podjęto próbę budowy modelu kalibracyjnego dla spektrometru VIS-NIR przeznaczonego do oznaczania koncentracji deoksyniwalenolu (DON) w ziarnie pszenicy ozimej już na etapie zbioru kombajnowego. W tym celu podczas żniw w sezonie 2011 zgromadzono w warunkach polowych materiał badawczy w postaci 14,5 tys. widm spektralnych absorpcji promieniowania oraz 283 prób ziarna. Niska koncentracja deoksyniwalenolu w naturalnych próbach ziarna nie pozwoliła na zbudowanie modelu kalibracyjnego. Dlatego opracowane modele PLS (R2≈0,9; RMSECV≈3 – 4 mg/kg) powstały w oparciu o 60 prób przygotowanych w warunkach laboratoryjnych. Zbiór modelowy złożony z prób o koncentracji DON w przedziale od 0 do 32 mg/kg został dodatkowo uzupełniony wynikami uzyskanymi dla 58 prób z naturalnych upraw. Walidacja modeli kalibracyjnych przeprowadzona w oparciu o widma spektralne zarejestrowane w warunkach polowych wykazała wyższą, jednak nadal niewystarczającą dokładność predykcji koncentracji DON za pomocą modelu zbudowanego w oparciu o oba zbiory prób.
Pracę zrealizowano w ramach projektu rozwojowego nr R12 0073 06 pt: „Opracowanie i walidacja technologii rozdziału strumienia ziarna podczas selektywnego zbioru zbóż" finansowanego przez MNiSW
***Słowa kluczowe**: bezpieczeństwo żywności, deoksyniwalenol, spektrometria VIS-NIR, model kalibracyjny PLS*

1. Introduction

Fusarium head blight is one of the most serious diseases affecting wheat worldwide. It is caused by *Fusarium graminearum* along with *F. culmorum*, *F. avenaceum* and other related fungi [3, 4, 10]. *Fusarium* species can infect host plants at the stage of seedling, heading and flowering - and consequently cause significant economic cereal crop losses [6, 12]. Cereals contaminated with *Fusarium* spp. and their mycotoxins e.g. deoxynivalenol (DON) [5, 7] are a risk to human and animal health.

The occurrence of *Fusarium* spp. in cereals of Polish origin, especially in wheat, represents a high risk of cereal contamination with toxic secondary metabolites and consequently, the toxicity of food and feed products. The occurrence of selected toxic metabolites in cereal kernels produced in some parts of Poland (Żuławy) was observed, where epidemics of wheat head blight appeared. *Fusarium* species and their toxic metabolites were identified there [11].

One of the methods of instrumental analysis which is widely applied in research on agricultural products is Near Infrared Spectroscopy (NIRS). This results from the fact of selective absorption of electromagnetic radiation by their basic components. The application of optical methods to detect fungal infections in cereal kernels, especially wheat, has gained wide interest, because in contrast to chemical methods, it is easier to make optical measurements and the results can be obtained in a shorter time and with a lower outlay [1, 8].

Studies [8] prove that it is possible to measure the content of DON higher than 60 ppm on the basis of NIR absorption spectrums. Further research enabled confirmation of the possibility to assess the DON content in kernels also

below that level [9]. This enabled selection of kernels depending on the DON content. On the other hand Beyer [2] observed that it is possible to detect diversified levels of DON content in samples containing more than 20% of *Fusarium* damaged kernels (FDK) even if the symptoms of infection are not visible with the naked eye.

Simultaneously, the aforementioned authors indicate that the highest variability of absorption of infrared radiation by samples containing FDKs with different degrees of infection can be observed within the wavelength from 1400 to1900 nm.

The abovementioned achievements inspired us to extend our research on selective harvesting of cereals by an attempt to evaluate the usefulness of VIS-NIR reflectance spectroscopy for detection of dangerous concentration of deoxynivalenol in wheat kernels as early as the stage of combine harvesting of winter wheat.

2. Material and methods

The research material in the form of spectrums and winter wheat kernel samples was collected during harvest in 2011. For the purpose a Claas Lexion 480 grain harvester was used. The machine was equipped with an AgroSpec spectrometer (tec5) and an automatic system of grain sample collection constructed at our Institute. The absorption spectrums of radiation were recorded with diffuse reflection within the wavelength ranging from 400 to 2170 nm with the interpolated resolution up to 2 nm. A measurement probe installed in the measurement channel accumulating the grain sample collected from the grain conveyor of the combine harvester was used for this purpose (Fig. 1). The measurement system was able to collect up to 7 grain samples at one time. The moment of sample collection was synchronised with the rate of its movement in the measurement channel. This was supposed to guarantee that the same grain portions would be analysed as those whose spectrums were recorded.

The research material was collected from 4 fields belonging to the experimental farms of Poznań University of Life Sciences. The fields, whose total area was 71.9 ha, were located in western Wielkopolska region (Poland). While the harvester was working 14.5 thousand spectrums were recorded and 283 grain samples were collected. 58 spectrums with the largest variability were selected from the spectrums recorded for the collected samples. The DON content was measured in the corresponding samples according to the following procedure.

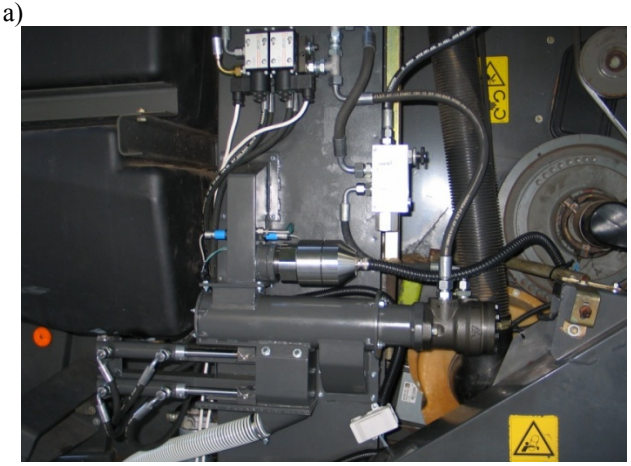
To analyse the trichothecene (DON), the extract obtained from crushed kernels was purified by filtration on a column (Celite 545, charcoal Darco G-60, neutral alumina, 3:9:5 w/w/w) conditioned with acetonitrile-water (82:18 v/v). DON was eluted with the same solvent. HPLC was carried out with a Waters-501 apparatus with C-18 Nova Pak column (3.9 mm x 300 mm) and a Waters 486 UV detector (λmax=224 nm). DON was eluted with 25% water solution of methanol (flow rate 0.7 ml·min^{-1}) after retention times of 11.72 minutes. The detection limit for DON was 0.01 mg·kg^{-1}. Positive results (on the basis of retention times) were confirmed by HPLC analysis with internal standards.

Simultaneously, in all of the collected samples moisture and protein content in the dry weight were measured with a Foss Infratec 1241 grain analyser.

Next, on the basis of the obtained results the first attempt to build the calibration model was made. Partial least squares regression (PLS) available in Unscrambler X software (CAMO Software AS) was used for this. Standard settings were used to build the calibration model: NIPALS algorithm, cross validation, 7 requested factors.

Unfortunately, the attempt ended in a failure due to a very low degree of natural *Fusarium* damage (Table 1, Column 1).

a)



b)

c)

Fig. 1. The measurement system installed in the combine harvester: a) the measurement channel, b) sample collection system, c) the computer and AgroSpec spectrometer (tec5) in the harvester cabin

For this reason new samples were prepared in a laboratory. Grain from crops grown under natural conditions was mixed with grain with visible signs of damage. FDKs were obtained from the plantations which were inoculated with *Fusarium culmorum*. There were 60 samples prepared so

that the variability of DON content would have equal distribution, ranging from 0 to 32 mg·kg^{-1}. The effect was achieved by preparation of samples containing from 0 to 10% of FDKs (1% variation) and then from 12 to 100% FDKs (2% variation).

As previously, the absorption spectrums of the prepared samples were recorded and then the DON content was measured in them. On the basis of those results two calibration models were built by means of PLS method.

The first of them (hereinafter called DON1) was built only on the basis of spectrums recorded for samples prepared in laboratory conditions. In the second model (DON2) the set model was applied, supplemented with absorption spectrums corresponding to the samples collected during harvest. The models built in that way were used to predict the DON concentration on the basis of the spectrums recorded in field conditions.

Due to the fact that the research presented in this study is conducted as part of a larger project concerning selective cereal harvesting the authors also tried to determine if the results concerning the DON content in winter wheat grain were anyhow connected with the content of protein the dry weight of grain. In order to do this Statistica 10 software was used to determine the Pearson linear correlation coefficient for the aforementioned data.

3. Results

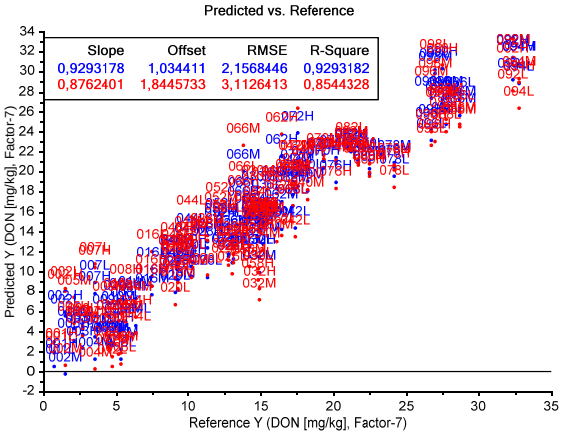
Figure 2 shows the diagrams presenting the correlations between the results of prediction and the reference results for the developed models.

The models were best matched with the model sets of data when the range of the spectrum under analysis was limited to 950-2030 nm and baseline conversion was applied. At that stage of the work it was possible to observe that the DON1 model, which was built exclusively on the basis of the data from laboratory samples, was characterized by slightly better data mapping. This fact is confirmed by relatively high value of the variance coefficient both for calibration ($R^2 = 0.92$) and prediction ($R^2 = 0.85$).

Unfortunately, for both models the high value of mean squared errors of calibration (RMSEC – blue in diagrams) and cross validation (RMSECV – red), which ranges from 2 to 4 mg·kg^{-1}, limits their application to indicate the exceeding of the acceptable limit of DON concentration in grain (1.25 mg·kg^{-1}). In spite of this fact action was taken to verify the values which will be returned by the above mentioned models on the basis of spectrums recorded in the conditions of combine harvesting of wheat. The prediction module from Unscrambler X software was used for that purpose. As a result two sets of data (for the DON1 and DON2 models) were obtained, which passed the Kolmogorov–Smirnov normality test with the application of the Lilliefors probabilities. This enabled verification of the models (DON1 and DON2) by comparison of the characteristic values describing each set with data for the set of results of laboratory analyses (Table 1).

The analysis of the results presented in the table confirms the fact that the models cannot be applied to predict the content of DON in wheat grain samples, because the error in the measurement of DON concentration in the samples is too high. Unfortunately, the additional analysis of the correlations between the contents of DON found in chemical analyses and the results of prediction for the spectrums corresponding to the samples sent to the laboratory also points to the absence of correlation between the real content of DON and the result of prediction. The obtained values of the Pearson correlation coefficient were -0.18 for the results obtained for the DON1 model and -0.17 for the prediction results obtained with the DON2 model.

a) DON1



b) DON2

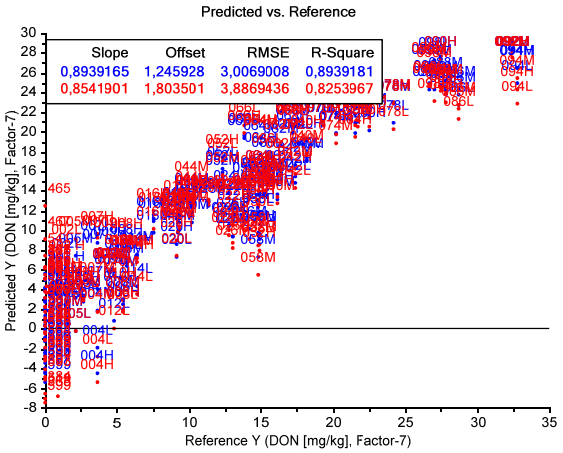


Fig. 2. The correlations between the results of prediction and reference values for the models: a) built only on the basis of laboratory samples, b) built on the basis of a model set supplemented with data from field investigations

Table 1. A comparison of the parameters characterizing the sets of prediction results with the parameters of the set of data from chemical analyses

Parameter	DON* mg·kg^{-1}	DON1 mg·kg^{-1}	DON2 mg·kg^{-1}
Mean	0.04	5.73	1.37
Standard deviation	0.13	8.30	3.32
Minimum value	0.00	-16.99	-12.43
Maximum value	0.91	29.26	8.75
Median	0.01	6.24	2.50
Mode	0.00	10.13	2.85
Cardinality	60	14.500	14.500

* Results of laboratory analyses

However, the parallel work on construction of the calibration model for measurement of protein content in the dry weight of wheat grain enabled observation of a definite negative correlation between the prediction results obtained from the DON1 model and the results of prediction of pro-

tein content. The Pearson correlation coefficient calculated for the aforementioned results on the basis of prediction from 21 212 spectrums recorded in field conditions was -0.42. This may suggest the fact that the DON1 model reacts not only to changes in the deoxynivalenol concentration in grain but it is also sensitive to changes in the protein content in the samples under analysis. This may have been caused by the method of preparation of the samples used for construction of the model. The gradual replacement of sound kernels with FDKs must have influenced the protein concentration in consecutive samples.

It is interesting to note that the aforementioned correlation cannot be observed for the values returned by the DON2 model. It is possible to suppose that the extension of the model set by the spectral data recorded for natural samples of wheat grain with different content of protein enabled reduction of the model sensitivity to variable protein concentration in the samples under analysis.

4. Conclusions

The low concentration of deoxynivalenol in the grain samples from natural plantations does not enable construction of the calibration model for the VIS-NIR spectrometer.

Although the application of artificially obtained samples of winter wheat grain containing a considerable percentage of kernels with visible symptoms of *Fusarium* infection enables construction of the calibration model that well reflects the DON concentration variability (R^2=0.85), the obtained RMSECV values are nearly three times as high as the acceptable limit of DON concentration in cereal grain (1.2 $mg \cdot kg^{-1}$). As a result it is impossible to apply the models to warn of the dangerous level of DON concentration during combine harvesting of wheat.

Although the supplementation of the model set with the spectral data recorded in field conditions deteriorates the determination coefficient (R^2=0.83) and RMSECV (about 3.9 $mg \cdot kg^{-1}$) obtained for the model, the values of DON concentration in grain returned by the model on the basis of the spectrums recorded in field conditions are burdened with a much smaller error. However, due to the error value it is still impossible to apply the model to indicate dangerous levels of DON concentration in wheat grain. For this reason the further course of the research will involve an attempt to build a classification model in three classes: sound kernels, kernels damaged to an acceptable level, kernels damaged to an unacceptable level.

Further research should also involve determination of the causes of the correlation between the results of prediction of the content of DON and protein in wheat grain.

5. References

[1] Balcerowska, G., Siuda, R., Sadowski, C.: Modelowanie zawartości materiału porażonego fuzariozą w rozdrobnionym ziarnie pszenicy na podstawie odbiciowych widm optycznych rejestrowanych w różnych zakresach długości fal. Acta Agrophyisca, 2008, 11(2), 323-334.

[2] Beyer, M., Pogoda, F., Ronellenfitsch, F. K., Hoffmann, L., Udelhoven, T.: Estimating deoxynivalenol contents of wheat samples containing different levels ofFusarium-damaged kernels by diffuse reflectance spectrometry and partial leastsquare regression. International Journal of Food Microbiology, 2010, 142, 370–374.

[3] Bottalico, A., Perrone, G.: Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. European Journal of Plant Pathology, 2002, 108, 611-624.

[4] Goliński, P., Kaczmarek, Z., Kiecana, I., Wiśniewska, H., Kaptur, P., Kostecki, M., Chełkowski, J.: Fusarium head blight of common polish winter cultivars – comparison of effects of Fusariumavenaceum and Fusariumculmorum on yield components. Journal of Phytopathology, 2002, 150, 135-141.

[5] Logrieco, A., Bottalico, A., Mule, G., Moretti, A., Perrone, G. Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. European Journal of Plant Pathology, 2003, 109, 645-667.

[6] Logrieco, A., Moretti, A., Mule, G., Paciolla, C., Ritieni, A.: Advances on the toxicity of the cereal contaminant Fusariumesadepsipeptides. Cereal Research Communications, 2008, 36, 303-313.

[7] Mesterhazy, A., Logrieco, A., Bailey, J. A., Corazza, L., Cooke, B. M.: Role of deoksynivalenol in aggressiveness of Fusariumgraminearum and F. culmorum and in resistance to Fusarium head blight. European Journal of Plant Pathology, 2002, 108, 675-684.

[8] Peiris, K. H. S., Pumphrey, M. O. & Dowell, F. E. NIR absorbance characteristics of deoxynivalenol and of sound and Fusarium-damaged wheat kernels. Journal of Near Infrared Spectroscopy, 2009, 17, 213-221.

[9] Peiris, K. H. S., Pumphrey, M. O., Dong, Y., Maghirang, Berzonsky, E. B. W., &Dowell, F. E. (2010). Near-infrared spectroscopic method for identification of Fusariumhead blight damage and prediction of deoxynivalenol in single wheat kernels. Cereal Chemistry, 2010, 87(6), 511-517.

[10] Srobarova, A., Slikova, S., Sudyova, V.: Diversity of the Fusarium species associated with head and seedling blight on wheat in Slovakia. Biologia, 2008, 63/3, 332-337.

[11] Tomczak, M., Wiśniewska, H., Stępień, Ł., Kostecki, M., Chełkowski, J., Goliński P.: Deoxynivalenol, nivalenol and moniliformin occurrence in wheat samples with scab symptoms in Poland (1998 – 2000).European Journal of Plant Pathology, 2002, 108(7), 625 - 630.

[12] Wiśniewska, H., Chełkowski, J.: Evaluation of susceptibility to Fusarium seedling blight in winter wheat cultivars, using digital image analysis. Plant Breeding and Seed Science, 1996, 40, 159-162.