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Assessment of pesticide coating on cereal seeds by near infrared hyperspectral imaging

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Classical chromatographic methods, such as ultra performance liquid chromatography (UPLC), are used as reference methods to assess seed quality and homogeneous pesticide coating of seeds. These methods have some important drawbacks since they are time consuming, expensive, destructive and require a substantial amount of solvent, among others. Near infrared (NIR) spectroscopy seems to be an interesting alternative technique for the determination of the quality of seed treatment and avoids most of these drawbacks. The objective of this study was to assess the quality of pesticide coating treatment by near infrared hyperspectral imaging (NIR-HSI) by analysing, on a seed-by-seed basis, several seeds simultaneously in comparison to NIR spectroscopy and UPLC as the reference method. To achieve this goal, discrimination—partial least squares discriminant analysis (PLS-DA)—models and regression—partial least squares (PLS)—models were developed. The results obtained by NIR-HSI are compared to the results obtained with NIR spectroscopy and UPLC instruments. This study has shown the potential of NIR hyperspectral imaging to assess the quality/homogeneity of the pesticide coating on seeds.

Keywords: cereal seeds, pesticide coating, NIR hyperspectral imaging

Introduction

Seed treatment with pesticides requires the active substances to be applied at the target rate and homogeneously distributed between seeds of the same batch. Indeed, a lower dose may lead to insufficient plant protection while an overdose can increase the risk of phytotoxicity. Nowadays, chromatographic methods, such as ultra performance liquid chromatography (UPLC), are the preferred reference methods for the quality control of the pesticide coating. They are selective, sensitive, accurate and repeatable, but also expensive, destructive and time consuming. Moreover, they require a substantial amount of solvent. Alternative methods that avoid these drawbacks are needed. In this context, near infrared (NIR) spectroscopy seems to be an interesting technique to control the quality of seed treatment. Several studies have shown the potential of vibrational spectroscopy to detect pesticide residues in food using NIR spectroscopy[1] and Raman[2] technology. The potential of NIR spectroscopy has also been demonstrated to classify active principles and to assess their concentration for the quality control of commercial pesticide formulations.[3] Other studies[4,5] have proved that NIR spectroscopy used with a seed-

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by-seed sample presentation allows the active substance concentration on the treated seeds to be quantified.

The objective of this study[6] was to show the potential of near infrared hyperspectral imaging (NIR-HSI) to assess the quality of pesticide coating treatment on cereal seeds. For this, five studies were performed, namely i) the identification of seed species, ii) the identification of the type of pesticide applied to the seeds, iii) the uniformity between seed batches based on the average dose of pesticides, iv) the consistency of treatment between seeds from the same batch based on the seed-by-seed dose and v) the homogeneity of the pesticide coating at the seed level. Discriminant chemometrics tools, namely partial least squares discriminant analysis (PLS-DA), and regression methods, partial least squares (PLS), were applied in order to characterise each of the studies. The results obtained by NIR-HSI were compared to the results obtained with classical NIR spectroscopy and UPLC.

Materials and methods

Seed samples

For this experiment, three cereal species (wheat, barley and spelt) were studied, as well as three groups of pesticide formulations characterised by three main active ingredients, prochloraz/triticonazole, prothioconazole and fludioxonil. In total, 87 samples were collected, most of them were coated directly by the seeds' producers and labelled, providing information on the pesticide formulation. Then two different studies were performed: in the first, in order to assess the homogeneity inter seed batches, all the samples were analysed in bulk (ring cup or eight seeds) using NIR-HSI (Burgermetrics, Latvia) as well as UPLC (Waters, USA) and NIR spectroscopy (Bruker-MPA, Germany) for comparison. In the second study, four samples, selected according to low or high pesticide dose, were analysed seed by seed. In total, 24 single seeds from each sample were analysed using UPLC and NIR-HSI in order to assess the homogeneity intra seed batches.

Reference method

Chromatographic methods are the reference methods used to assess the pesticide content. In this study, analyses were performed using ultra performance liquid chromatography (UPLC, Waters, USA) in order to quantify the active ingredients (prochloraz/triticonazole and prothioconazole). The active ingredient content is expressed in g of active ingredient per 100 kg of seed, taking into account the weight of the seed bulk for the analysis of the average dose or the weight of the seed for the analysis of the seed-by-seed dose. For the group treated with prochloraz/triticonazole, the sum of each active ingredient was calculated. The final results were expressed in % of the target dose which are 16 g/100 kg (12 g/100 kg for prochloraz + 4 g/100 kg for triticonazole) and 10 g/100 kg for prothioconazole.

NIR hyperspectral imaging and chemometrics

Hyperspectral images were collected using an NIR hyperspectral line scan instrument combined with a conveyor belt. All images consisted of lines of 320 pixels that were acquired at 209 wavelength channels (1100–2400 nm). This instrument is described in detail by Vermeulen *et al.*[7]

The image treatment involved building libraries for each class of species, treated/not treated status and group of pesticides. To extract the data from the image, a mask to isolate the seeds was built. The first step consisted of detecting and removing the pixels/spectra in the

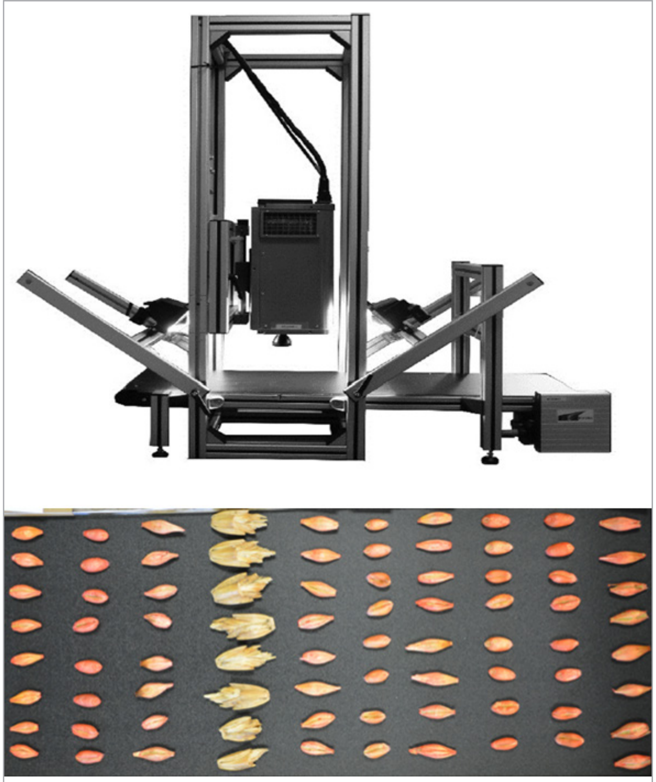

Figure 1. NIR hyperspectral imaging system and multi seeds presentation to the conveyor belt (eight seeds per sample).

Table 1. Performance of the PLS-DA equations discriminating the T/NT status of the seeds bulks according to the species using NIR-HSI in comparison to NIR spectroscopy.

NIR spectroscopy							
		Wheat		Barley		Spelt	
		T	NT	T	NT	T	NT
Calibration	n_c	48	3	14	3	2	2
	T	100	0	100	0	100	0
	NT	0	100	0	100	0	100
Cross-validation	T	100	0	100	0	100	0
	NT	0	100	0	100	0	100
Validation	n_v	8		4		1	
	T	100		100		100	
	NT	0		0		0	
NIR hyperspectral imaging							
		Wheat		Barley		Spelt	
		T	NT	T	NT	T	NT
Calibration	n_c (×8 seeds)	48	3	14	3	2	2
	T	97	4	89	17	100	7
	NT	3	96	11	83	0	93
Cross-validation	T	96	4	87	17	94	14
	NT	4	96	13	83	6	86
Validation	n_v (×8 seeds)	8		4		1	
	T	97		87		100	
	NT	3		12		0	

n_c, n_v: number of samples for calibration and validation, respectively; T: Treated; NT: not treated

image that showed a saturation of the absorbance corresponding to the conveyor belt. Then, the density-based spatial clustering of applications with noise (DBSCAN) method was applied to study the neighbourhood of the pixels detected as cereal seed.[8] This technique is one of the most common clustering algorithms. It groups together points that are closely packed together, i.e. with many nearby neighbours, and marking as outliers all those points that lie alone in low-density regions, i.e. whose nearest neighbours are too far away. After application of DBSCAN, for each defined cluster (seed), the mean spectra were calculated and used to build the libraries.

Various chemometric methods were then applied in order to extract the maximum amount of information from the spectral data. The unsupervised technique of principal component analysis (PCA) was used to obtain information on the natural separation between spectra. Then two supervised approaches were developed: one to perform classification of seed samples using PLS-DA using the predefined groups as reference and another to perform quantitative analysis of pesticide applied on the seeds using PLS and with UPLC values as reference.[9] In order to validate the models built, around 80% of samples were selected for model construction (calibration set) and 20% as validation set. The samples were selected in order to browse the variability in terms of pesticide coating and varieties. The number of samples is presented in Tables 1 and 2 according to the developed models. All the spectra were pre-processed using standard normal variate (SNV) and first derivative Savitzky–Golay (window = 5, polynomial = 2). The results of the discrimination models are expressed in different ways. Sensitivity refers to the percentage of samples from the class studied that have been correctly classified by the model. Specificity refers to the percentage of samples not from the class studied that have been correctly classified

Table 2. % pesticide target dose and % underdosing and overdose of barley seeds batches using UPLC, NIR spectroscopy and NIR-HSI.

		% Target dose						% Samples		
Groups of pesticide	Active ingredient	*n*	mean	*sd*	min	max	*cv*	<30%	>30%	±30%
Ultra performance liquid chromatography										
Group 1	Prochloraz + triticonazole	2	73.9	8.4	68.0	79.9	11.4	50	0	50
Group 2	Prothioconazole	5	52.1	15.1	34.2	66.9	29.0	100	0	100
NIR spectroscopy										
Group 1	Prochloraz + triticonazole	2	58.9	19.2	45.3	72.4	32.5	50	0	50
Group 2	Prothioconazole	5	58.1	19.0	40.1	83.2	32.7	60	0	60
NIR hyperspectral imaging										
Group 1	Prochloraz + triticonazole	2	59.3	24.4	42.1	76.6	41.1	50	0	50
Group 2	Prothioconazole	5	48.4	14.5	32.4	66.1	30.0	100	0	100

n: Number of samples; *sd*: standard deviation; *cv*: coefficient of variation

by the model. The performance of the PLS models is assessed in terms of the *RMSEP* (root mean square error of prediction) and the *RPDP* (ratio between the standard error on reference values of the validation set and the *RMSEP*). All data treatment was carried out using Matlab 7.5.0 (R2007b) and the PLS toolbox 7.0.2.[9]

Results and discussion

PLS-DA discrimination models

NIR spectra of treated and not treated seeds from wheat, barley and spelt obtained with a hyperspectral camera are presented in Figure 2. The main differences between species are that the seed husk is thicker in spelt and barley than wheat kernels, and within the wavelength range between 1650 nm and 1800 nm there are differences in relation to the cellulose and starch content.[10] The differences between treated and not treated seeds within each species are less identifiable on raw spectra.

As a first step, the unsupervised technique PCA was applied to the data to get some indication about the natural grouping of the seeds. PCA was able easily to distinguish the different species (wheat, barley and spelt) and the treated/not treated (T/NT) status of the seeds. PCA also highlighted some trends to distinguish three groups of pesticide in relation to the main active ingredient. Based on this information, in a second step, a dichotomist classification tree could be built where each node of the tree corresponded to a PLS-DA model for a specific group of seeds.[11] In a first level, a PLS-DA model discriminating the three species was built; then, as second level, for each of them, a PLS-DA model discriminating the T/NT status of the seeds was constructed and finally, as third level, for the treated set, a PLS-DA model discriminating the three groups of pesticides applied to the seeds was also developed. Models were developed

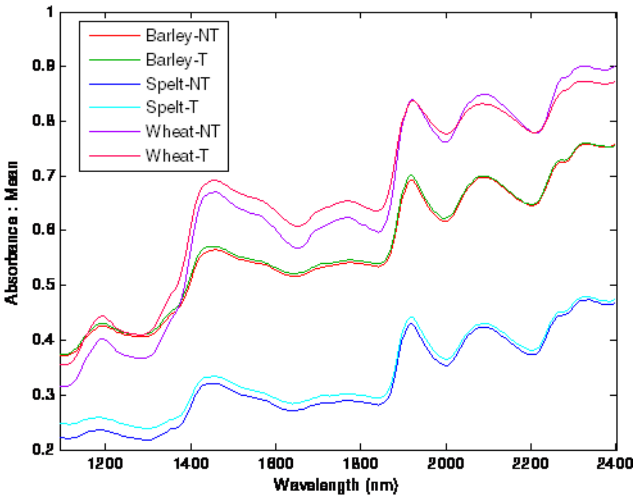

Figure 2. NIR-HSI spectra of treated (T) and not treated (NT) seeds from wheat, barley and spelt.

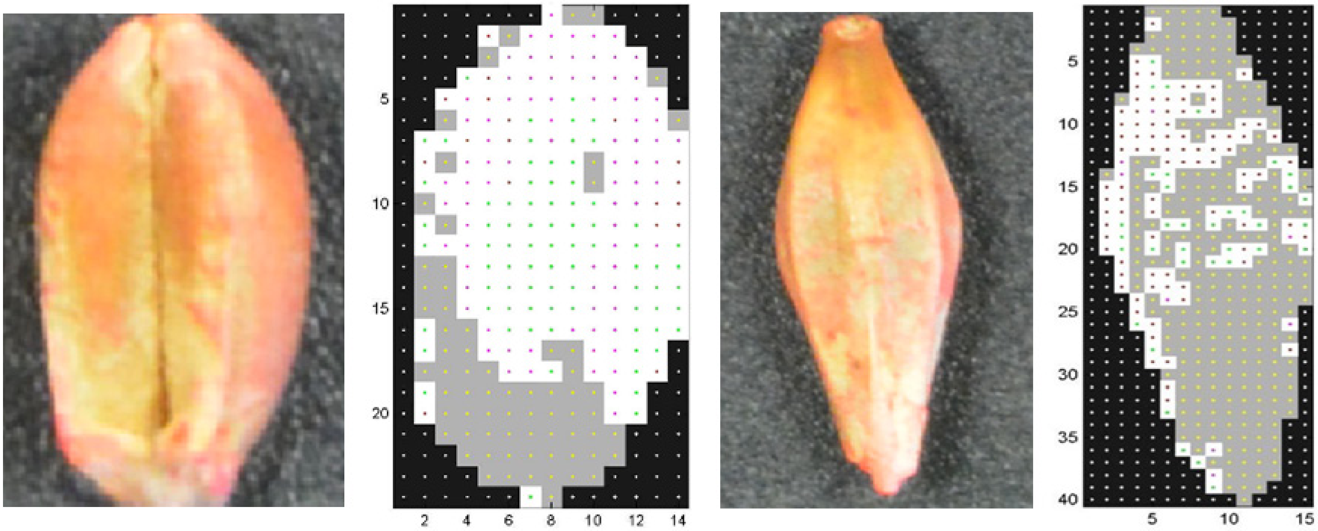

Figure 3. RGB pictures and predicted images showing on single seeds (wheat and barley) pixels detected as treated in white and pixels detected as untreated in grey.

on mean spectra by sample for the NIR spectroscopy data and on mean spectra by seed for the NIR-HSI data.

PLS-DA models allow classification of the seed bulks according to the species (wheat, barley and spelt) with a sensitivity of 100% whichever the instrument (NIR or NIR-HSI). Regarding the T/NT status, Table 1 shows the performance of the PLS-DA equations. In comparison to NIR spectroscopy (sensitivity = 100%), the sensitivity using NIR-HSI is lower and comprised between 87% and 100% in validation (model at seed level). The discrimination according to the type of pesticide allows differentiating the groups with a sensitivity between 75% and 100% using NIR spectroscopy and between 25% and 93% using NIR-HSI (results not shown).

Compared to NIR spectroscopy, NIR hyperspectral imaging provides additional information on the presence of cereal seeds mixture in a batch, and on the presence of untreated or not correctly treated seeds in a treated seeds batch. Figure 3 shows RGB pictures and corresponding predicted NIR images for one wheat seed and one barley seed not correctly coated with pesticide. Pixels detected as treated using the PLS-DA model are represented in white and pixels detected as untreated in grey.

PLS regression models

PLS regression models were built using UPLC to determine the reference values in order to assess the quantity of pesticide applied to the seeds. The PLS model built from barley seed batches treated by different active ingredients produced, for the validation set, a *RMSEP* of 2.95 (*RPDP* = 1.28) using classical NIR spectroscopy and of 3.49 (*RPDP* = 1.08) for NIR-HSI. The PLS model built from seed-by-seed produced using NIR-HSI, for the validation set, a *RMSEP* of 2.34 and 6.75 g of active ingredient per 100 kg of seed, and a *RPDP* = 1.67 and 1.45 on barley and wheat, respectively. Based on these results, the regression PLS models developed in this study allow classifying the treated seeds into two groups: underdosed and overdosed. According to the guidelines provided by the Pesticide Safety Directorate (PSD), the dose applied to the seeds should be ±30% around the targeted dose.[12] Therefore, the two groups were defined as follows: underdosing (<30%) and overdose (>30%). Table 2 shows the % target dose results obtained on barley seeds batches treated by two different active ingredients, using UPLC, NIR spectroscopy and NIR-HSI. 50% of seed batches treated with prochloraz and triticonazole were detected as underdosed whatever the method used. For the seeds batches treated with prothioconazole, 100%, 60% and 100% of seeds batches were underdosed using the three methods, respectively.

Table 3 shows the % target dose results obtained on wheat/barley single seeds sets using UPLC and NIR-HSI. For the wheat samples 1 and 2, 37% and 100% of the seeds were overdosed or underdosed using UPLC and 21% and 96%, respectively, using NIR-HSI. For the barley samples 3 and 4, 87% and 71% of the seeds were over-

Table 3. % pesticide target dose and % underdosing and overdose of wheat/ barley single seeds sets using UPLC and NIR-HSI.

		% Target dose						% Seeds		
	Sample	*n*	mean	*sd*	min	max	*cv*	<30%	>30%	±30%
Ultra performance liquid chromatography										
Wheat	1	24	86.60	30.30	35.80	159.00	37.60	33	4	37
	2	24	239.70	147.50	143.70	795.70	61.50	0	100	100
Barley	3	24	49.60	30.50	23.10	149.20	61.60	83	4	87
	4	24	55.20	27.50	22.00	107.20	50.00	71	0	71
NIR hyperspectral imaging										
Wheat	1	24	93.60	26.30	40.70	165.60	28.10	12	8	21
	2	24	187.30	35.70	115.20	269.40	19.00	0	96	96
Barley	3	24	44.50	22.10	8.50	103.50	49.60	87	0	87
	4	24	49.50	25.00	17.10	93.70	50.40	79	0	79

n: Number of samples; *sd*: standard deviation; *cv*: coefficient of variation

dosed or underdosed using UPLC and 87% and 79%, respectively, using NIR-HSI.

Conclusions

The application of chemometrics to NIR hyperspectral images offers new prospects for the quality control of the coating efficiency of pesticides on seeds. The study shows that PLS-DA models allow sorting the seeds based on the species (i), on the treated/untreated status and in some cases on the type of pesticide (ii). On another hand, the regression models allow classifying the treated seeds into two groups according to the average dose of pesticides: underdosing and overdose (iii). This methodology allows also the pesticide coating homogeneity between seeds inside one batch to be assessed (iv), and also the pesticide coating homogeneity at the seed level to be assessed (v).

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