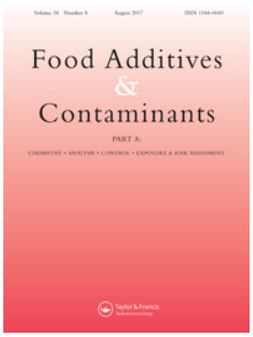

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ARTICLE

Online detection and quantification of particles of ergot bodies in cereal flour using near-infrared hyperspectral imaging

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ABSTRACT

The objective of this study is to assess near-infrared (NIR) hyperspectral imaging for the detection of ergot bodies at the particle level in cereal flour. For this study, ground ergot body samples and wheat flour samples as well as mixtures of both from 100 to 500,000 mg kg^{-1} were analysed. Partial least squares discriminant analysis (PLS-DA) models were developed and applied to spectral images in order to detect the ergot body particles. Ergot was detected in 100% of samples spiked at more than 10,000 mg kg^{-1} and no false-positives were obtained with non-contaminated samples. A correlation of 0.99 was calculated between the reference values and the values predicted by the PLS-DA model. For the cereal flours containing less than 10,000 mg kg^{-1} of ergot, it was possible for some samples spiked as low as 100 mg kg^{-1} to detect enough pixels of ergot to conclude that the sample was contaminated. However, some samples were under- or overestimated, which can be explained by the lack of homogeneity in relation to the sampling issue and the thickness of the sample. This study has demonstrated the potential of NIR hyperspectral imaging combined with chemometrics as an alternative solution for discriminating ergot body particles from cereal flour.

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Ergot; contaminant; alkaloid; cereal; feed; food; NIR hyperspectral imaging; multivariate imaging analysis

Introduction

For decades, near-infrared (NIR) spectroscopy has been widely used in the food and feed sectors for the rapid implementation of inexpensive and efficient control tools to assess the quality of products. In the 2000s, in the context of the bovine spongiform encephalopathy (BSE) crisis, NIR combined with a hyperspectral imaging system has been proposed for the detection of animal ingredient particles in feedstuffs (Fernández et al. 2004; Baeten & Dardenne 2005; Vermeulen et al. 2010). More recently, this technology was investigated as a means of detecting undesirable and toxic contaminants in cereal flour intended for the food and feed sectors (Vermeulen et al. 2013a; Mishra et al. 2015).

In this direction, one important concern is the presence of ergot sclerotia formed by the fungi *Claviceps purpurea*, containing toxic alkaloids. The European Commission (EC) has set a limit of 1000 mg kg^{-1} for ergot in all feedstuffs containing unground cereals (EC 2002). This limit has been set at 500 mg kg^{-1} for ergot bodies in cereals for humans (EC 2015) in order to reduce the risk of poisoning. The official classical physical determination of the contamination rate of cereals by ergot may not be sufficient, as the content of alkaloids can vary significantly from one sclerotia to another (EFSA 2012). Recent studies showed that the alkaloid content in ergot can variate from 57 to 36,385 µg g^{-1} in France (Orlando & Piraux 2014) and from 1 to 6003 µg g^{-1} in the Netherlands (Mulder et al. 2012). In 2012, the EC adopted a recommendation inviting member states to provide chemical methods to monitor simultaneously the sclerotia content and the ergot alkaloids in the samples (EC 2012). In response to this legislation, screening methods have been developed for the online detection and quantification of ergot sclerotia using NIR hyperspectral imaging (Vermeulen et al. 2013b). Concerning the detection of ergot fragments, the International Association of Feedingstuff Analysis (IAG) has provided an elegant early warning tool using light microscopy. With this official method,

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ergot fragments from the sieved fractions sized less than 0.5 mm can be easily identified by colouring, using a solution of ethanol and sodium hydroxide (IAG 2011). However, this method is time consuming. The objective of the study reported here is to demonstrate the potential of NIR hyperspectral imaging for the detection of ergot at the particle level in cereal flour.

Materials and methods

Samples

Twenty-six different samples were used: ground ergot body coming from different sources (wheat and rye) and wheat flour samples of different compositions (white, semi-whole, whole or bran flours). Moreover, several mixtures of wheat white flour and ergot particles were prepared. Table 1 describes the samples and gives their respective ergot concentration in mg kg^{-1}, and for some samples their alkaloid content in µg kg^{-1} was analysed by the chromatographic method.

Pure samples

Three batches of ergot sclerotia were collected: one from wheat production in Belgium and two sets collected in France from wheat and rye productions respectively. Each batch of sclerotia was ground using a grinder (IKA A11) and sieved in order to obtain ergot powder of two sizes: < 1 and > 1 mm. Six samples (E1–E6) of pure ergot powder were prepared in this way.

In addition, four wheat flours (W1–W4) were bought in a supermarket: one wheat white flour, one whole wheat flour, one semi-whole wheat flour and one wheat bran flour.

Mixtures of wheat–ergot

A first set of 11 samples (MA1–MA11) of wheat contaminated with ergot from 0% to 2.6% was used. These samples were prepared by Arvalis in the framework of an inter-laboratory study (unpublished data) dedicated to assess the performance of the methods used by the subcontractors (Figure 1). A total alkaloids content from 10 to 57,884 µg kg^{-1} of alkaloids was determined by a reference method. The remainder of the samples were collected for this study. The average concentration of total ergot alkaloids in sclerotia was 2298 µg g^{-1}. This value is consistent with the average total content, generally assumed to be around 2000 µg g^{-1} for the 12 major alkaloids (Mulder et al. 2012).

A second set of five samples of 50 mg (MC1–MC5) was prepared and mixed manually by sprinkling ergot powder in wheat flour. They include five proportions of wheat–ergot (25–25, 40–10, 45–5, 47.5–2.5, 49.5–0.5 mg) corresponding to respectively 50%, 20%, 10%, 5% and 1% of ergot. This sample size was chosen in relation to the quantity of sample analysed by NIR hyperspectral imaging.

Reference method

Visual observations combined with confirmation by light microscopy are the reference method for ergot contamination in grains (EC 2008). This method was used to confirm that the black bodies used in the preparation of the mixtures are really ergot sclerotia. These bodies were weighed in order to know the ergot sclerotia content in the sample. Chromatographic methods are the reference methods to assess the alkaloid content (Krska & Crews 2008). The first set of 11 samples spiked at a low level of ergot concentration were analysed using this method by five European laboratories in the framework of an inter-laboratory study. For each laboratory, each sample was subjected to extraction in acetonitrile. The mixture was shaken on a flatbed shaker and then filtered. An aliquot was transferred to a glass test tube and mixed with the internal standard solution. Samples were shaken with primary-secondary amine (PSA) for solid-phase extraction (SPE), and aliquots were transferred with a syringe and filtered into vials. Ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine and their corresponding epimers (-inine forms) were analysed by LC-MS/MS. The sum of these 12 alkaloids was calculated for each laboratory. For each sample, the average of each of the five laboratories is retained for the study. A correlation of 0.99 was obtained between the alkaloid content obtained by LC-MS/MS and the ergot sclerotia content weighed for the mixtures preparation.

NIR hyperspectral imaging

A NIR hyperspectral imaging system with a conveyor belt (BurgerMetrics SIA, Riga, Latvia) was used. This instrument is described in detail by Vermeulen et al. (2012). The lens was set up in this

Table 1. Description of the samples used and results of ergot detection in wheat.

		Reference values		Predicted values							
				Wheat flour	Ergot						Sub-images
		Ergot	Alkaloids	Pixels	Pixels	Minimum	Maximum	Mean	SD	CV	with ergot
Sample code	Description	mg kg^{-1}	µg kg^{-1}	nb	nb	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	nb/20
W1	Wheat white flour	0		115,310	0	0	0	0	0	n.a.	0
W2	Whole wheat flour	0		113,757	0	0	0	0	0	n.a.	0
W3	Semi-whole wheat flour	0		122,950	0	0	0	0	0	n.a.	0
W4	Wheat bran flour	0		120,233	0	0	0	0	0	n.a.	0
MA1	Mixture wheat–ergot set 1	0	10	121,327	1	0	177	9	40	4.2	1
MA2	Mixture wheat–ergot set 1	10	31	124,850	4	0	482	34	112	3.3	2
MA3	Mixture wheat–ergot set 1	130	280	124,693	45	0	4.015	379	1.030	2.7	5
MA4	Mixture wheat–ergot set 1	320	673	122,069	0	0	0	0	0	n.a.	0
MA5	Mixture wheat–ergot set 1	640	1.419	122,638	15	0	796	129	241	1.9	6
MA6	Mixture wheat–ergot set 1	1,030	2.257	124,112	107	0	8.475	897	2.268	2.5	6
MA7	Mixture wheat–ergot set 1	1,280	2.965	123,864	6	0	330	51	118	2.3	3
MA8	Mixture wheat–ergot set 1	3,210	7.261	121,018	3	0	330	26	80	3.1	2
MA9	Mixture wheat–ergot set 1	6,410	13.935	122,821	0	0	0	0	0	n.a.	0
MA10	Mixture wheat–ergot set 1	12,820	28.272	124,435	200	0	7.896	1.558	2.222	1.4	13
MA11	Mixture wheat–ergot set 1	25,640	57.884	110,059	474	0	23.845	4.503	6.616	1.5	16
MC1	Mixture wheat–ergot set 2	10,000		123,556	6.382	1.782	158.575	45.451	41.699	0.9	20
MC2	Mixture wheat–ergot set 2	50,000		134,114	5.940	2.910	150.755	41.310	36.859	0.9	20
MC3	Mixture wheat–ergot set 2	100,000		142,125	5.903	5.680	99.853	40.510	22.496	0.6	20
MC4	Mixture wheat–ergot set 2	200,000		115,748	14.163	15.462	155.302	71.448	36.846	0.5	20
MC5	Mixture wheat–ergot set 2	500,000		39,485	73.864	99.853	855.670	652.780	157.299	0.2	20
E1	Ergot (wheat-B < 1 mm)	1,000,000		0	83.120	1,000,000	1,000,000	1,000,000	0	0	20
E2	Ergot (wheat-B > 1 mm)	1,000,000		0	74.306	1,000,000	1,000,000	1,000,000	0	0	20
E3	Ergot (wheat-Fr < 1 mm)	1,000,000		115	61.549	978.783	1,000,000	998.005	5.315	0.005	20
E4	Ergot (wheat-Fr > 1 mm)	1,000,000		110	82.448	972.946	1,000,000	998.576	6.049	0.006	20
E5	Ergot (rye-Fr < 1 mm)	1,000,000		0	62.863	1,000,000	1,000,000	1,000,000	0	0	20
E6	Ergot (rye-Fr > 1 mm)	1,000,000		1	108.449	999.834	1,000,000	999.991	37	0.00004	20

nb: number; n.a.: not applicable.

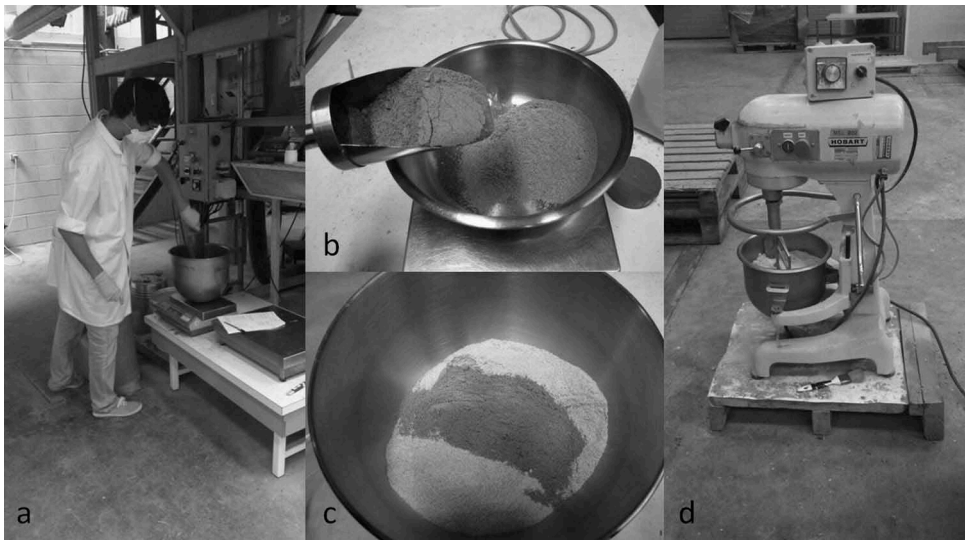

Figure 1. (a) Weighing of whole wheat flour (0.8 mm), (b) weighing of ergot powder (0.5 mm), (c) the mixture between ergot powder and wheat flour, and (d) the homogenisation of the mixture.

case to analyse 1 cm width on the conveyor belt. The sample was spread on the spectralon support, using a sieving device. The sample covered an area around 1 cm^2 and had a thickness of about 1 mm (Figure 2). NIR spectra (1118–2425 nm) were recorded in reflection mode by co-adding 32 scans per pixel (30 × 30 µm). An image of 320*400 spectra was recorded per powder sample.

Data treatment

The first step involved building libraries for ergot particles and wheat flour particles by selecting around 800 pixels from six images with ergot particles (E1–E6) and four images with wheat particles (W1–W4). A model was then developed using PLS-DA (Wise et al. 2006). The model was built using three latent variables and using leave-one-out cross-validation to optimise the model. It showed a sensitivity (a measure of how often the model correctly identified a positive sample as positive) and a specificity (a measure of how accurate the model was against false-positives) of 100% for the two classes (wheat flour and ergot).

The equation built could be used to predict new images from unknown samples. When performing the prediction of a new image (Figure 3), the following procedure was applied:

- Detection and elimination of pixels/spectra in the image showing a spike in relation to a dead pixel (indicated by a black line).
- Detection and elimination of pixels/spectra in the image showing a saturation of the

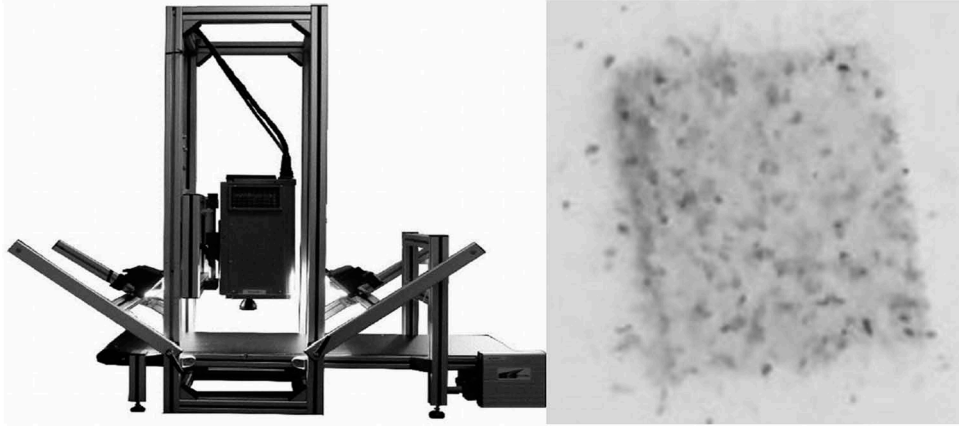

Figure 2. Burgermetrics near-infrared (NIR) hyperspectral imaging system and cereal flour adulterated with ergot on spectralon.

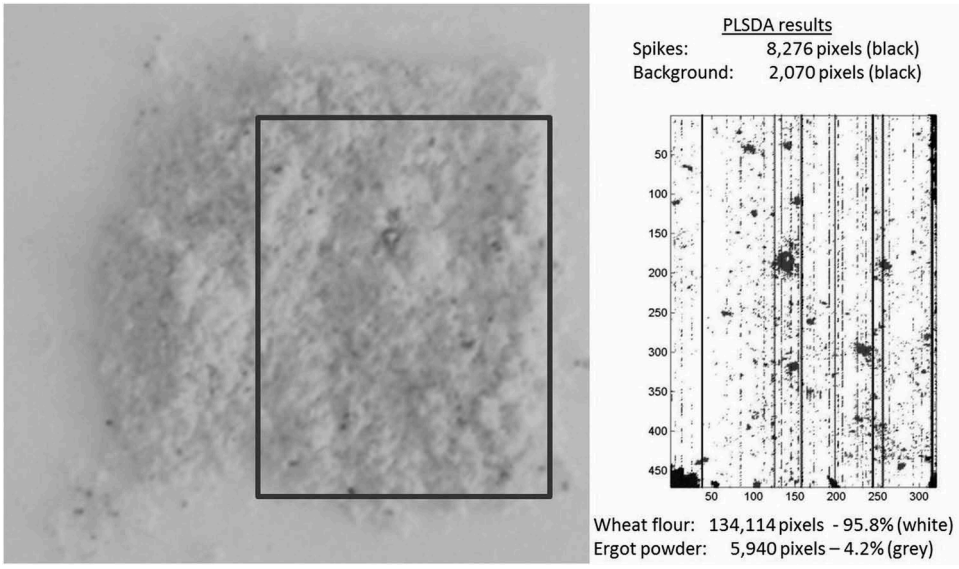

Figure 3. (l eft) RGB picture and (right) predicted near-infrared (NIR) hyperspectral image showing spikes (black line), background (black), ergot particles (grey) in a wheat flour (white) sample adulterated with 5% ergot powder.

absorbance corresponding to the conveyor belt (indicated in black).

- Detection of pixels/spectra detected as wheat by PLS-DA model (indicated in white).
- Detection of pixels/spectra detected as ergot bodies by PLS-DA model (indicated in grey).

All the data analysis was performed using Matlab R2007b (The Mathworks Inc., Natick, MA, USA). It allows one to obtain for each predicted image information about the number and percentage of pixels detected for each class (spikes, background, wheat flour and ergot powder) (Figure 3).

Quantification of ergot particles

In order to determine the quantity of ergot in a sample, the ratio between the number of pixels detected as ergot and the sum of the pixels detected as wheat and ergot together is calculated and expressed in mg kg^{-1}.

Results and discussion

NIR hyperspectral imaging results

To assess the potential of the NIR hyperspectral imaging system combined with the PLS-DA classification method, the model built from pure wheat and ergot samples was applied to the two sets of samples of wheat contaminated with ergot. Table 1 shows, beside the description and the reference values, the values predicted by applying the PLS-DA model. For each sample, Table 1 gives the number of pixels detected as wheat and ergot as well as the proportion of pixels detected as ergot in the samples, expressed in mg kg^{-1} (mean). Figure 4 shows the correlations

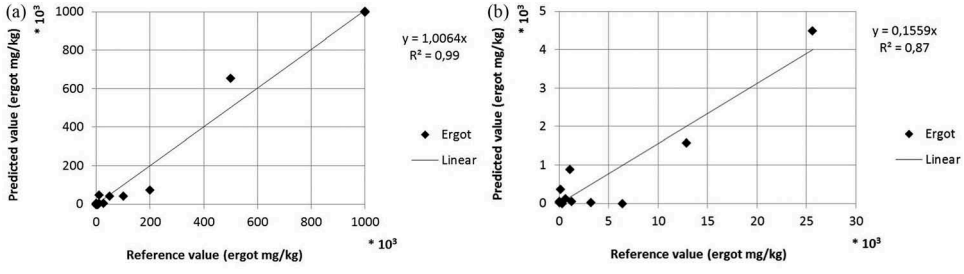

Figure 4. Correlation between the reference values and the predicted values expressed in mg kg^{-1} of ergot using partial least squares discriminant analysis (PLS-DA) applied to near-infrared (NIR) hyperspectral images of (a) the 26 samples and (b) the 11 samples at low ergot concentration (< 30,000 mg kg^{-1}).

between the reference values and the predicted values expressed in mg kg^{-1} of ergot using PLS-DA applied to NIR hyperspectral images. Correlations of 0.99 and 0.88 were obtained respectively when the 26 samples of the study or only the 11 samples of set 1 at low ergot concentration (0–2.6%) were taking into account.

The results presented in Table 1 and Figure 4 show 0.05% of false-negative results for pure ergot powder samples and no false-positives results for pure wheat flours. For a wheat sample containing a low level of ergot contamination, it was possible to detect enough pixels of ergot to conclude that the sample was contaminated. For instance, it was shown that the cereal flour sample MA6, spiked with 1030 mg kg^{-1} (0.1%) of ergot (i.e., the maximum ergot level for livestock feed; EC 2002) was predicted to contain 897 mg kg^{-1} using NIR hyperspectral imaging. These results are in agreement with previous studies using this technology for the detection of other types of contaminants. Fernández Pierna et al. (2014) showed that melamine could be detected in soybean meal samples adulterated at levels as low as 0.1%. Mishra et al. (2015) showed that peanuts could be detected in wheat flour samples at levels as low as 0.01%.

Homogeneity study

To assess the homogeneity of the sample (50 mg), the images of 320*400 pixels were divided into 20 sub-images of 80*80 pixels. Each sub-image corresponds to the analysis of a subsample of 2.5 mg. Table 1 shows for each sample the statistical parameters (minimum, maximum, mean, SD and coefficient of variation (CV)) regarding the proportion of pixels detected as ergot using the PLS-DA model and expressed in mg kg^{-1}. The number of sub-images by sample including predicted ergot is also displayed. For the unspiked wheat samples (W1–W4), no false-positive results were detected for all the 20 sub-images of the four samples and the ergot concentration was predicted as 0 mg kg^{-1}. For the slightly spiked samples (MA1–MA11), ergot was detected in 0–16 sub-images amongst the 20 sub-images of each sample, according to the level of adulteration. No ergot was detected in 75% of sub-images. The CV calculated for each sample was higher than 1. In wheat sample MA6 with an ergot contamination at 1030 mg kg^{-1} and a predicted ergot content of 897 mg kg^{-1}, the predicted values for the 20 sub-images varied between 0 and 8475 mg kg^{-1}. For the samples spiked at more than 1% (MC1–MC5), ergot was detected in all the 20 sub-images of each sample. The CV lay between 0.24 and 0.92. In wheat sample MC2 with an ergot contamination level at 50,000 mg kg^{-1} and a predicted ergot content of 41,310 mg kg^{-1}, the predicted values for the 20 sub-images varied between 2910 and 150,755 mg kg^{-1}. For the ergot samples (E1–E6), ergot was also detected in all the 20 sub-images of each sample. The CV was below 0.01. Wheat flour particles were detected in six sub-images amongst the six samples.

From these results, we can conclude that for samples at low level of adulteration (< 50,000 mg kg^{-1}), the sampling is crucial. For samples spiked above 50,000 mg kg^{-1}, a sub-sample of 2.5 mg is enough as ergot is detected in the 20 sub-images. For samples spiked between 10,000 and 50,000 mg kg^{-1}, 50 mg should be analysed and for lower adulteration, more than 50 mg should be sampled.

As it can be observed in Table 1 that some samples were underestimated, while others were overestimated. This can be explained by the reduced portion of samples analysed (sampling issue) and by lack of homogeneity of the sample, as described in the previous section. Coefficients of variation from 0.2 to 4.2 were calculated for the spiked samples.

Zengling et al. (2016) also showed the complexity in detecting melamine if the particles are under or tightly embedded in the soybean meal particles and if the thickness of the layer is more than 100 μm. Particle size, surface roughness and density of the sample spread on a spectralon surface in a single layer are also important factors in quantifying contaminants correctly.

Conclusions

The analysis by NIR spectroscopy of wheat flour and ergot powder (Vermeulen et al. 2016) confirmed the typical spectra of wheat grains and ergot sclerotia obtained by previous studies (Vermeulen et al. 2011), which ensure that the two matrices can be discriminated. However, the PLS-DA models developed on the NIR spectra were unable to discriminate wheat flours spiked with low concentrations of ergot

particles (Vermeulen et al. 2016). To perform such analysis at a particle level, systems such as NIR probe or NIR microscopy (NIRM) are needed in order to select the suspicious particles. A study performed by Ebene (2016) showed the feasibility of detecting ergot particles in wheat flours using NIRM.

From the results obtained using NIR hyperspectral imaging, we can conclude that ergot was detected in 100% of samples spiked at more than 10,000 mg kg^{-1} and that no ergot was detected in any of the blank samples. A correlation of 0.99 was calculated between the reference values and the predicted values. For the cereal flours containing less than 10,000 mg kg^{-1} of ergot, it was possible for some samples spiked as low as 100 mg kg^{-1} to detect enough pixels of ergot to conclude that the sample was contaminated. However, some samples were under- or overestimated. This can be explained by lack of homogeneity and the thickness of the sample.

This study has demonstrated the potential of NIR hyperspectral imaging combined with chemometrics as a relevant solution for discriminating ergot body particles from cereal flour.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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