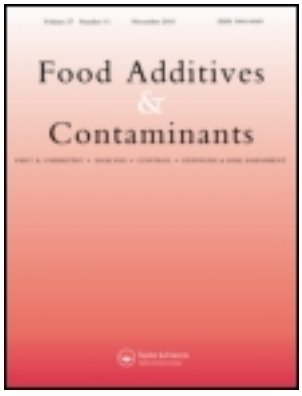

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Online detection and quantification of ergot bodies in cereals using near infrared hyperspectral imaging

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The occurrence of ergot bodies (sclerotia of *Claviceps purpurea*) in cereals presents a high toxicity risk for animals and humans due to the alkaloid content. To reduce this risk, the European Commission fixed an ergot concentration limit of 0.1% in all feedstuffs containing unground cereals, and a limit of 0.05% in 'intervention' cereals destined for humans. This study sought to develop a procedure based on near infrared hyperspectral imaging and multivariate image analysis to detect and quantify ergot contamination in cereals. Hyperspectral images were collected using an NIR hyperspectral line scan combined with a conveyor belt. All images consisted of lines of 320 pixels that were acquired at 209 wavelength channels (1100–2400 nm). To test the procedure, several wheat samples with different levels of ergot contamination were prepared. The results showed a correlation higher than 0.99 between the predicted values obtained using chemometric tools such as partial least squares discriminant analysis or support vector machine and the reference values. For a wheat sample with a level of ergot contamination as low as 0.01 %, it was possible to identify groups of pixels detected as ergot to conclude that the sample was contaminated. In addition, no false positives were obtained with non-contaminated samples. The limit of detection was found to be 145 mg/kg and the limit of quantification 341 mg/kg. The reproducibility tests of the measurements performed over several weeks showed that the results were always within the limits allowed. Additional studies were done to optimise the parameters in terms of number of samples analysed per unit of time or conveyor belt speed. It was shown that ergot can be detected using a speed of 1–100 mm/s and that a sample of 250 g can be analysed in 1 min.

Keywords: ergot; contaminant; alkaloid; cereal; feed; food; NIR hyperspectral imaging; multivariate imaging analysis

Introduction

Many species of mycotoxigenic fungi colonise grain cereals, leading to the contamination of the cereals and cereal products with mycotoxins. Among the fungal species affecting cereals during the flowering period is *Claviceps purpurea*. This fungus grows parasitically on some grasses and on several cereal crops, such as rye, wheat and barley. It produces sclerotia or ergot bodies, small banana-shaped blackish resting stages, which can replace grain kernels in the head of grain. During harvest, many sclerotia fall to the ground where they overwinter and germinate the following spring, with many small mushrooms. Others remain between the grain kernels during threshing and can end up in grain products. Ergot bodies can contain a mixture of alkaloids that have powerful pharmacological properties. Ergot alkaloids are associated with human and animal illnesses. Ergotism, a human disease, was known in Europe's Middle Ages as 'St. Anthony's fire' or 'holy fire'. Gangrenous symptoms in medieval outbreaks of ergotism in humans were described; early symptoms included hallucinations, swollen limbs and a burning sensation in the limbs, followed by necrosis leading to the loss of limbs (CAST 2003a). The disease is caused by eating products made from ergot-contaminated grains. Outbreaks of human ergot poisoning have occurred several times over the past century (CAST 2003a).

Ergot alkaloids also cause animal diseases, including bovine abortion and intestinal inflammation in sheep (IPCS 1990); ergot poisoning is a constant danger in livestock rearing (CAST 2003b). The European Food Safety Authority (EFSA) published a scientific report on ergot, describing it as an undesirable substance in animal feed (EFSA 2005). The EFSA concluded that the concentration of alkaloids in the ergot bodies is variable (0.01 and 0.21%) and that a consistent relationship between the amount of sclerotia

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and the total ergot alkaloid concentration cannot be established. This conclusion was confirmed by Scott (2009), who reviewed data on the concentrations of alkaloids in sclerotia, reporting ranges of 0.011–0.45% in rye ergots, 0.013–0.31% in wheat ergots, 0.082–1.04% in barley ergots and 0.042–0.74% in triticale ergots. For directly monitoring of ergot alkaloids in food and feed commodities, chromatographic methods have been mainly used. Several reviews of the different methodologies for detecting ergot alkaloids have been published by Scott (2005, 2009) and Krska and Crews (2008). Some of the methodologies are based on multiplex dipstick assays (CONffIDENCE 2011). None of the current analytical methods for ergot alkaloids has been formally validated in inter-laboratory studies, probably because the legal limits for individual ergot alkaloids in food and feed are not known (FAO 2004). Regulatory analyses are, therefore, seldom carried out, thus limiting the need for validated methods. Although there are currently no EU-regulations for ergot alkaloids in food and feed, these could be established in the near future, depending on a forthcoming scientific report on the risks to human and animal health of ergot alkaloids in food and feed that EFSA is preparing at the request of the European Commission (EC). The report should, *inter alia*, update the earlier report on ergot in feed (EFSA 2005).

The regulatory situation in the EU for ergot bodies is different from the situation described above for ergot alkaloids. European directive 2002/32/EC on undesirable substances in animal feed fixed a limit of 0.1% (1000 mg/kg) for ergot in all feedstuffs containing unground cereals (EC 2002), to reduce the risk of poisoning. EEC regulation 689/92, which sets out the procedure and inspecting cereals conditions for intervention agencies in charge of governing the cereals European market, trade with third countries and rules regarding competition, limited the concentration of ergot bodies in cereals for humans to 0.05% (500 mg/kg) (EC 1992). A survey of the presence of undesirable botanic substances in feed, carried out in 2006 in official control laboratories in all EU Member States, showed a resurgence of the presence of ergot bodies in cereal samples (van Raamsdonk et al. 2009). EC regulation 687/2008 (EC 2008) placed ergot in the 'miscellaneous impurities' category. This regulation states that the official method for detecting ergot bodies in cereals is to pass a 250 g cereal sample through several sieves, spread 50–100 g of the main fraction (1–3.5 mm) on a table, and then use tweezers to extract the impurities, including the ergot sclerotia. This method is similar to that proposed in the French regulation NF EN 15587 (AFNOR 2009). Alternative methods include the microscopy method proposed by the International Association of Feedingstuff Analysis (IAG), which provides an elegant early warning tool for ergot contamination, including ergot fragments. With this method, ergot bodies and fragments larger than 0.5 mm in at least 250 g of the feedstuff are determined by macroscopic identification. Sieve fractions less than 0.5 mm are identified by colouring, using a solution of ethanol and sodium hydroxide. However, this method is time-consuming (IAG 2011).

The current study proposes an alternative method for detecting and quantifying ergot bodies in cereals using an online NIR hyperspectral imaging system combined with some chemometric tools (Baeten and Dardenne 2005; Baeten et al. 2007, 2010; Vermeulen et al. 2010, 2011). This technique is faster than the classical microscopic method and enables a large quantity of material to be analysed, thereby avoiding the sampling problems associated with representative sampling. The following sections describe the technique and the experiments performed to assess the performance of the NIR hyperspectral imaging system.

Material and methods

Samples

Seven samples (S1–S7) consisting of 320 g of wheat contaminated with 0, 100, 500, 1000, 1500, 5000 and 10,000 mg/kg were prepared. The average weight of one ergot body was 0.032 g. An additional sample (S8) was prepared for validation purposes. This sample contained 20 g of wheat mixed with seven selected ergot bodies (a ¼ ergot body, a ½ ergot body and five full ergot bodies of varying lengths and widths). The weight ratio of the mixture was 17,200 mg/kg ergot. Table 1 describes the samples.

NIR hyperspectral imaging system

Hyperspectral images are collected using an NIR hyperspectral line scan or push-broom imaging system, combined with a conveyor belt (BurgerMetrics SIA, Riga, Latvia). The instrument used for this study is an SWIR XEVA CL 2.5 320 TE4 camera from XENICS using an ImSpector N25E spectrograph that includes a cooled, temperature-stabilised mercury–cadmium–telluride (MCT) detector from SPECIM Ltd. (Oulu, Finland). The system projects a beam of light onto a two-dimensional focal plane array (FPA) and each image consists of 320-pixel lines acquired at 209 wavelength channels: 1100–2400 nm at 6.3 nm intervals with 32 scans per image. The resulting images provide a reflectance spectrum for each pixel. The acquisition is done

Table 1. Description of the samples used to assess the potential of the methods.

Sample code	Ergot bodies (nb)	Ergot bodies (mg/kg)
S1	0	0
S2	1	100
S3	5	500
S4	10	1000
S5	15	1500
S6	50	5000
S7	100	10,000
S8	7	17,200

using HyperProVB software (BurgerMetrics SIA, Riga, Latvia).

Prior to analysis, the spectral imaging system was calibrated with a dark image (by blocking the lens entrance) and a white image (background) collected from a standard white reference board (empty teflon plate). The spectra were then automatically corrected accordingly. This procedure was performed to compensate for offset due to the dark current, the light source temperature drift and the lack of spatial lighting uniformity.

The conveyor belt speed is fixed at 3 mm/s (i.e. 20 lines/s) to produce clear images. A complete study was done, however, to optimise the speed, taking into account the quantity of cereals being analysed. The lens is set up to cover 10 cm of the width of the conveyor belt. Thus, 6400 spectra (20×320) covering an area of 3 cm^2 (± 12 seeds) were acquired each second (i.e. ± 530 spectra per seed).

Data treatment

Hyperspectral data provide an interesting opportunity for developing new processing techniques. Multivariate image analysis (MIA) techniques have been developed in recent years mainly for classifying images into categories, as have techniques for detecting similar materials and extracting information at the pixel scale (Amigo et al. 2008; Geladi 2008). In this paper, chemometric tools based on MIA techniques were applied for the data treatment.

The first step involved building libraries for ergot bodies, wheat and background by selecting pixels for two images, one with ergot and the other with wheat, taken before sample analysis. Models were then developed using two chemometric tools, partial least squares discriminant analysis (PLSDA) (Wise et al. 2006) and the most recent technique – support vector machines (SVM) (Fernandez et al. 2004). PLSDA is a classical linear technique often used due to its simplicity, speed and good performance. For the optimisation of the PLSDA model, leave-one-out cross validation was carried out to find the number of variables showing the best classification rate (or lower error). SVM has the ability to model nonlinear relationships by projecting, in a non-linear way, the data from the original space to a feature space of higher dimension where the separation between data becomes linear. For the optimisation of the SVM model, a grid-search technique was used to find the optimal parameters driving to the best classification rate. The equations built could be used to predict new images or to perform online prediction directly for the new samples. When performing the prediction of an image of new samples (Figure 1a), the following procedure was applied:

(1) Detection and elimination of pixels/spectra in the image showing a saturation of the absorbance corresponding to the conveyor belt (indicated in black in Figure 1b).
(2) Detection and elimination of pixels/spectra detected as wheat by PLSDA/SVM models (indicated in black in Figure 1c).
(3) Detection of pixels/spectra detected as ergot bodies by PLSDA/SVM models (indicated in grey in Figure 1c).
(4) Application of the density-based spatial clustering of applications with noise (DBSCAN) method (Daszykowski et al. 2001) to study the neighbourhood of the pixels detected as ergot bodies in step (3). Using this technique, pixels within 2 pixels of each other and with a minimum of 12 neighbour pixels were agglomerated into a single class and identified as an ergot body (indicated in white in Figure 1d). Pixels that did not meet these conditions were identified as outliers (indicated in grey in Figure 1d). This technique was not applied when the PLSDA model was performed online using proprietary software.

Quantification of ergot bodies

Two ways of quantifying ergot in the samples were applied to provide either the proportion of pixels detected as ergot bodies in the samples (mg/kg Ergot) or the number of ergot bodies in the samples (Nb Ergot).

The proportion of pixels detected as ergot (mg/kg Ergot) in the samples is determined by calculating the ratio between the number of pixels detected as ergot and the sum of the pixels detected as wheat and ergot together. The number of ergot bodies (Nb Ergot) corresponds to the number of clusters obtained using

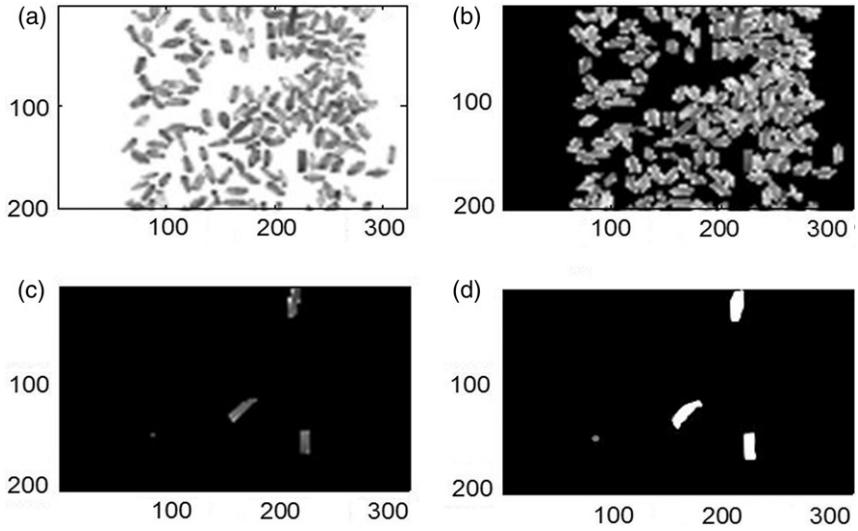

Figure 1. Ergot prediction in wheat using the SVM model showing one image capture of 200 * 320 pixels for (a) the hyperspectral image at 1721 nm, (b) the image 'a' after removing the pixels detected as spectra belonging to the conveyor belt, (c) the image 'b' after removing the pixels detected as spectra belonging to wheat, (d) the image 'c' after applying DBSCAN. Grey indicates pixels erroneously detected as ergot, white clusters are groups of pixels detected as ergot bodies.

the DBSCAN technique or to the number of ergot kernels in the images counted visually.

Results and discussion

Comparison of PLSDA and SVM classification methods

To assess the potential of the NIR hyperspectral imaging system combined with the two classification methods described here (PLSDA and SVM), the two models built from pure wheat and ergot samples were applied to the seven samples of wheat contaminated with 0–10,000 mg/kg of ergot. Table 2 shows the results obtained by applying the PLSDA and SVM models. For each sample, the table gives the number of ergot bodies detected and the percentage of pixels detected as ergot bodies calculated from the number of pixels detected as ergot or wheat. Figure 2 shows the correlations between the predicted values obtained using the PLSDA or SVM models and the reference values; for both the number of ergot bodies and for the proportion of ergot criteria. In all cases, a correlation higher than 0.99 was obtained. Using the PLSDA model, the coefficients of correlation obtained were 0.9995 and 0.9997 for the number and percentage of ergot bodies, respectively; using SVM, similar results were obtained (0.9998 and 0.998, respectively).

The results presented in Figure 2 and Table 2 show that for a wheat sample containing a level of ergot contamination as low as 100 mg/kg, it was possible to detect enough pixels of ergot (310 pixels using the PLSDA model; 119 pixels using the SVM model) to conclude that the sample was contaminated. Conversely, for samples without ergot, not enough pixels of ergot (65 pixels using the PLSDA model; 0 pixels using the SVM model) were detected to reach a conclusion that the sample was contaminated.

Using the PLSDA model, no false negatives were found, but 0.005% of false positives were identified (Sample S7). Using the SVM model, no false negatives were found, but 0.011% of false positives were identified (Samples S3, S4, S7). Most of the false positives resulted from wheat germ pixels detected as ergot pixels. When the results were expressed in ergot numbers, the predicted value correlated well with the reference value. When the results were expressed in ergot proportion, the predicted value was overvalued by 10–50%. It should be noted that, for the reference value, the ergot proportion is a weight ratio, whereas for the predicted value it is an area ratio. Because the area of the ergot bodies used in this study was about the same as the wheat kernels, and because the thousand-kernel-weight (TKW) was 32 g for the ergot bodies and 40 g for the wheat kernels, the predicted value needed to be corrected by a factor of 0.8 (32/40).

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) is the minimum ergot concentration that can be detected by the technique used. It is generally agreed that the LOD starts with a

Table 2. Results of ergot body detection in wheat.

	PLSDA				SVM			
Sample code	Ergot bodies (nb)	Wheat pixels (nb)	Ergot pixels (nb)	Ergot proportion (mg/kg)	Ergot bodies (nb)	Wheat pixels (nb)	Ergot pixels (nb)	Ergot proportion (mg/kg)
S1	0	1,165,764	65	56	0	1,146,910	0	0
S2	1	1,175,247	310	264	1	1,178,066	119	101
S3	5	1,201,068	840	699	6	1,205,466	760	630
S4	10	1,186,307	1501	1264	11	1,183,884	1582	1336
S5	15	1,308,104	2257	1722	15	1,299,021	2556	1968
S6	50	1,260,232	7804	6154	50	1,243,547	9244	7434
S7	103	1,246,731	16,844	13,330	104	1,238,406	18,694	15,095

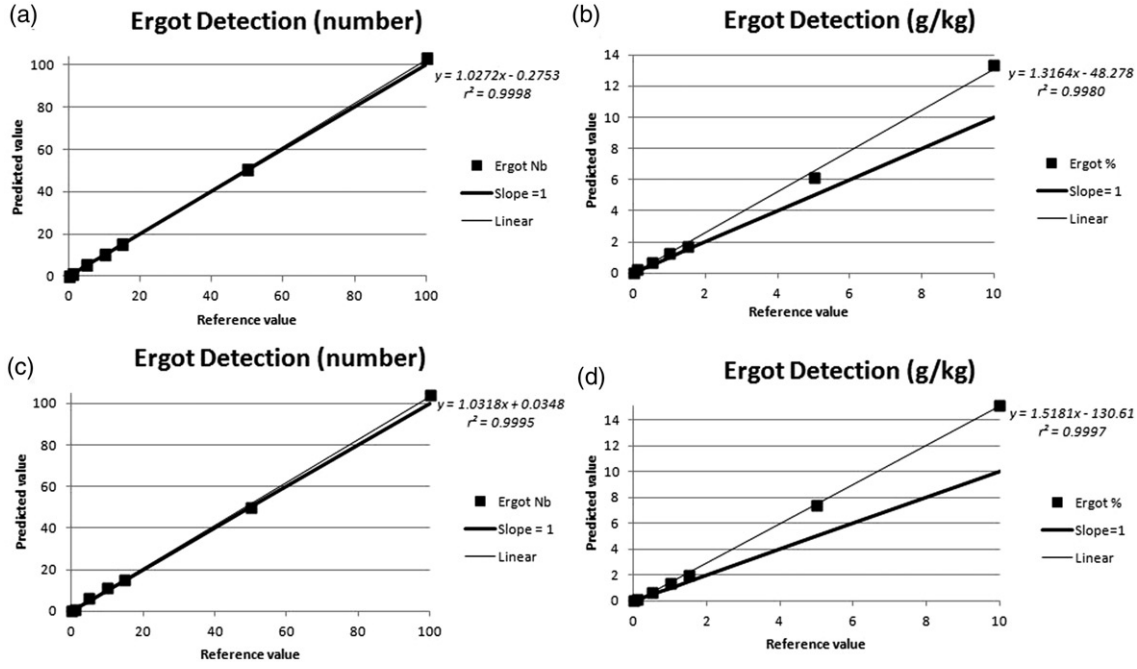

Figure 2. Results expressed in ergot body numbers (a–c) or percentage (b–d), showing the correlation between the quantity of ergot bodies counted visually and the quantity detected using the PLSDA model (a–b) or the SVM model (c–d) in wheat samples with 0–1% ergot contamination level.

concentration equal to 3 standard deviations of the blank (Thomsen et al. 2003). EU legislation fixed the authorised concentrations of ergot bodies in 'intervention' cereals, for humans and for all feedstuffs containing unground cereals, at 500 and 1000 mg/kg, respectively. The limit of quantification (LOQ) is the minimum concentration of ergot that can be quantified using the technique. It is generally agreed that quantification starts with a concentration equal to 10 standard deviations of the blank (Thomsen et al. 2003).

To determine the LOD and LOQ, a test was performed by measuring 20 times the wheat samples contaminated with 0 and 500 mg/kg of ergot (S1 and S3). Table 3 shows, for both samples and for each measurement, the number of pixels detected as ergot or wheat using the PLSDA model, as well as the proportion of ergot expressed in mg/kg. For a wheat sample with an ergot contamination of 0 mg/kg, the results varied between 20 and 101 mg/kg of pixels detected as ergot; for a wheat sample with an ergot contamination level of 500 mg/kg, the results varied between 322 and 877 mg/kg of pixels detected as ergot.

In this experiment, 0% of false negatives and 0.001 % of false positives were detected. The false positive cases (samples S8 and S15) were linked to the

Table 3. Results of the LOD and LOQ assessment.

	S1 0 mg/kg–0 ergot			S3 500 mg/kg–5 ergots		
Analyses (repetition)	Wheat pixels (nb)	Ergot pixels (nb)	Ergot concentration (mg/kg)	Wheat pixels (nb)	Ergot pixels (nb)	Ergot concentration (mg/kg)
1	1,279,014	28	22	1,297,336	431	332
2	1,275,878	68	53	1,300,798	419	322
3	1,268,860	95	75	1,298,922	608	468
4	1,269,386	105	83	1,300,093	424	326
5	1,261,160	92	73	1,299,901	567	436
6	1,261,022	96	76	1,293,750	605	467
7	1,266,354	97	77	1,292,903	563	435
8	1,263,728	121	96	1,292,653	607	469
9	1,211,020	105	87	1,289,450	587	455
10	1,267,397	112	88	1,295,548	642	495
11	1,254,999	93	74	1,299,255	543	418
12	1,262,381	128	101	1,300,504	478	367
13	1,261,548	107	85	1,289,635	484	375
14	1,285,128	84	65	1,289,405	580	450
15	1,278,307	47	37	1,230,348	598	486
16	1,273,892	49	38	1,284,717	709	552
17	1,271,539	38	30	1,288,563	709	550
18	1,266,532	25	20	1,285,456	772	600
19	1,262,162	29	23	1,296,446	1138	877
20	1,263,038	31	25	1,282,145	1060	826
Minimum	1,211,020	25	20	1,230,348	419	322
Maximum	1,285,128	128	101	1,300,798	1138	877
Average	1265167	78	61	1,290,391	626	485
SD	14,759	35	28	15,246	188	146
CV	0.01	0.45	0.45	0.01	0.3	0.3

Note: SD: Standard Deviation; CV: Coefficient of variation.

classification of an elongated part of two wheat kernels (19 and 21 pixels) as ergot.

From these results, we can conclude that ergot was detected in 100% of samples adulterated at 500 mg/kg (485 ± 146 mg/kg) and that no ergot was detected in any of the blank samples. From the data on the unadulterated samples, the noise could be estimated as the average of the pixels detected as ergot $\pm$ the standard deviation (SD) (i.e. 61 ± 28 mg/kg). The LOD was estimated to be 145 mg/kg ($61 + 3 \times 28$ mg/kg). The LOQ was estimated to be 341 mg/kg ($61 + 10 \times 28$ mg/kg). These results indicated that the LOD and LOQ of the methodology were easily capable of meeting the current EU regulatory limits for cereals.

Control chart

To test the ability of the method to give the same results for detection when the analysis was repeated day after day, two control charts were constructed using sample S8 as the reference sample. This sample was measured twice a day. The discrimination model SVM built for developing the method was applied to determine the number of pixels detected as ergot or wheat and to calculate the ratio between them. Over 1 month, 29 measurements were made. The first 14 measurements were used to calculate the SD and the average value for the percentage of ergot; the next 15 were used to validate the control chart. Overall, 1.5% of false negatives and 0.014% of false positives were detected. The cases of false negatives resulted from grouping together two ergots close to each other in one cluster using DBSCAN.

According to ISO 12099:2010 (ISO 2010), ± 2 SD (95% probability) and 3 SD (99.8% probability) are warning and action limits, respectively. On the control chart (Figure 3), the warning limits ± 2 SD ($\pm 0.26\%$) are drawn in small dots, the action limits ± 3 SD ($\pm 0.40\%$) in large dots and the black line represents the average value for the percentage of ergot (2.14%). To perform the analyses, the percentage of ergot calculated has to be within the action limits. If the calibration is performed correctly, only 1 of 20 points should plot outside the warning limits and 2 of 1000 points outside the action limits. The control chart in

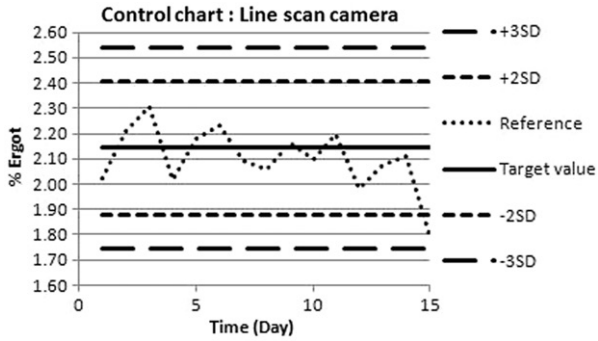

Figure 3. Control chart for ergot detection using the SVM models.

Figure 3 indicates that, for the 15 measurements performed for the validation, one sample was extreme but still within the action limits.

According to NF EN 15587, the repeatability limit should be assessed according to the ergot concentration in a sample, using this equation:

$$r = 2.8 * ((0.06 * \ \%\text{ergot}) + 0.12) = 0.70\%$$

Following this protocol, the 15 measurements performed for the validation were always within the limits allowed. Note that NF EN 15587 (limit $[r] = 0.7\%$) is not as strict as ISO12099-2010 (limit $[\pm 3SD] = 0.4\%$).

These results show that the method gives the same result when the same sample is analysed day after day.

Conveyor belt speed study

With the push-broom imaging system, the conveyor belt speed is an important parameter to take into account. The test was performed using the sample S7 analysed at conveyor belt speeds from 1 to 100 mm/s and a conveyor belt width of 10 cm.

For each conveyor belt speed, Table 4 shows the time taken analysing 250 g of cereals, the quantity of cereals analysed per hour, the number of pixels detected as ergot or wheat using the PLSDA model, and the ergot concentration as a percentage. It also shows that the higher the conveyor belt speed, the shorter the analysis time and the lower the number of pixels detected as ergot, and that the percentage of ergot was at the same level (1.15–1.83%).

These results indicate that a 250 g sample could be analysed in 1 min using the push-broom imaging system, with a 50 mm/s conveyor belt speed and a 10 cm belt width. Up to 37 kg of cereals could be analysed using this system at a speed of 100 mm/s over 1 h. The performance could be improved using a set-up covering the full belt width (30 cm), which would mean up to 111 kg (3×37 kg) in 1 h. By comparison, the time taken to analyse 250 g using the macroscopic observation method (EC 687/2008, NF EN 15587) and the microscopy method (IAG) would be about 15 and 60 min, respectively (IAG 2010).

The results also show that, at a speed of 100 mm/s, it was possible to detect enough pixels of ergot (539) to conclude that the sample was contaminated at a ratio of 1.54%. For a 3 mm/s conveyor belt speed, the ratio was 1.41%. This can be compared with the results in Table 2 for the same speed and the same sample: the ratio obtained was 1.33 %. As noted earlier, a correction factor of 0.8 should be applied to address the fact that the result is expressed as an area ratio, and not a weight ratio. By applying this correction, the ergot fraction evaluation changes from 1.41 to 1.12%.

Conclusions

One of the most important applications of NIR hyperspectral imaging technology is likely to be in the processing industry, where accurate and fast inspection is needed. This study has demonstrated that this technology can be used to detect and quantify ergot contamination in cereals destined for food or feed, both in the laboratory or in the production chain using online prediction. In this particular case, and in line with European legislation, it was shown that 500 mg/kg (the maximum ergot level for human food) and 1000 mg/kg (the maximum ergot level for livestock feed) can be detected using the NIR imaging system. This method enables a 250 g sample to be analysed in 1 min using the push-broom imaging system. Using a set-up based on the full conveyor belt width (30 cm) and a belt speed of 100 mm/s, up to about 100 kg grain/h could be analysed, whereas the current microscopy method requires 15–60 min to analyse 250 g. It should be noted that the NIR imaging technique has been developed for detection of ergot bodies based on existing EC regulations but it will not be suitable for possible future new EU regulations based on ergot alkaloid limits for which destructive instrumental analysis will be still required.

One of the biggest challenges with using this kind of technology is the need for fast data-processing equipment to process the images. In this study, two chemometric-based methods, PLSDA and SVM, were successfully applied. In the experiments, the number of false positives obtained using these techniques was 0.005% (0.001–0.01 %). Most of the false positives resulted from wheat germ pixels detected as ergot pixels. The false negative rate was 0.5% (0–1.5 %). Most of the false negatives resulted from the

Table 4. Results of analysis of a sample contaminated with ergot at 1% w/w, according to conveyor belt speed and using the PLSDA model.

Conveyor belt speed (mm/s)	Time to analyse 250 g (min)	Quantity analysed/h (kg/h)	Wheat pixels (nb)	Ergot pixels (nb)	Ergot concentration (%)
1	41.9	0.4	3,408,039	39,769	1.15
2	21.3	0.7	1,688,149	25,182	1.47
3	14.6	1	1,127,107	16,137	1.41
4	10.3	1.5	842,665	12,178	1.42
5	8.5	1.8	671,636	10,941	1.6
6	6.7	2.2	556,737	8923	1.58
7	6.6	2.3	478,234	8526	1.75
14	3.3	5	240,316	4401	1.8
20	2.2	7	240,083	4148	1.7
30	1.6	9	111,467	1980	1.75
40	1.1	14	84,101	1518	1.77
50	1	16	66,766	1194	1.76
60	0.8	18	55,506	1007	1.78
70	0.7	22	48,773	884	1.78
80	0.6	26	42,373	791	1.83
90	0.5	31	38,006	691	1.79
100	0.4	37	34,435	539	1.54

non-distinction of two ergot bodies close to each other. More work on this technology is needed. The next step will be some comparative and validation studies. For this, real samples from industry will be used for validation of the proposed protocol and a complete study of method performance between this new technology and the microscopic method will be performed. Another step could be the development of similar models for the simultaneous detection and quantification of impurities or other contaminants of plant, animal and mineral origin, as well as for determining quality and nutritional parameters. To develop a perfect system for detecting contaminants, however, it is important to remember that the spectra in a pixel are often a mixture of the spectra of the pure constituents, and that spectral signatures of various sources, where the signatures have not already been analysed, might be present.

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