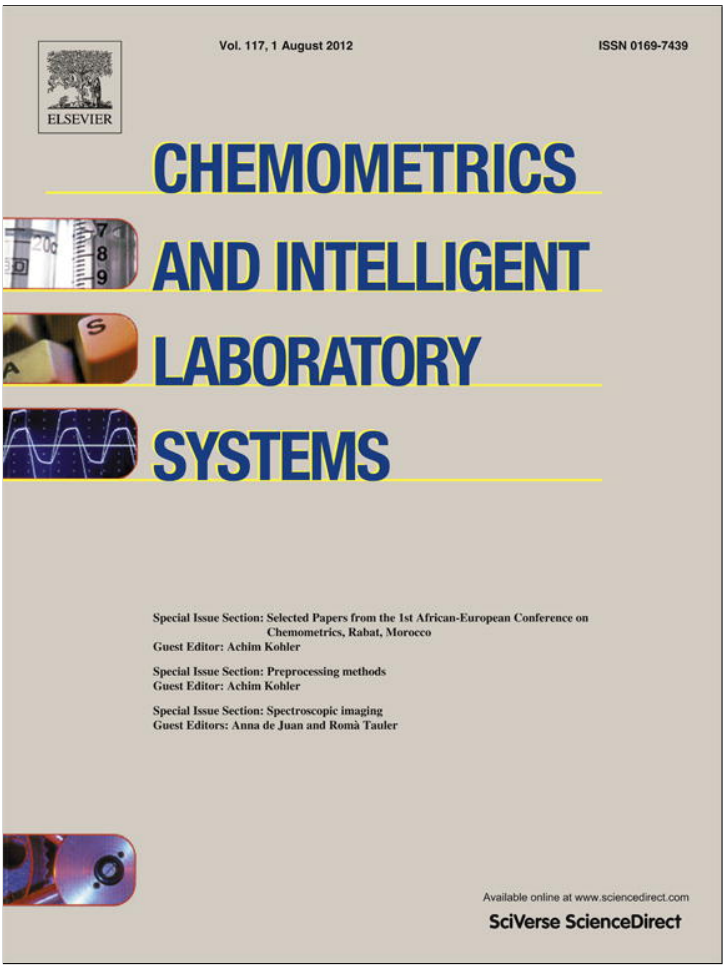

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NIR hyperspectral imaging spectroscopy and chemometrics for the detection of undesirable substances in food and feed

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ABSTRACT

Food and feed safety as well as quality control analyses are often carried out using reference methods that have limitations in terms of adequation for the optimum implementation at the different steps of the food/feed chain and for the control of the end-products. Recent developments in analytical instrumentation and data processing methods have led to increased use of spectroscopic techniques, being proposed to establish alternative methods replacing the reference techniques. In recent years, these improvements have included the development of NIR hyperspectral imaging methods combined with appropriated chemometric tools. The research aim of this paper is to show that combining NIR hyperspectral imaging spectroscopic technique with chemometrics can greatly improve food and feed safety and quality control. For this purpose, two case studies were conducted using two different NIR hyperspectral imaging systems combined with chemometric tools and spectral rules applied in a dichotomist way. The first study focused on the detection of impurities in cereals in order to integrate a complete methodology into an automatic cereal selection or production chain. The second study focused in the contamination of plants by pathogens and showed the potential of this combination for detecting and quantifying cyst nematodes in sugar beet roots.

1. Introduction

Food and feed safety as well as quality control analyses are often carried out using reference methods (wet chemistry) that have limitations in terms of adequation for the optimum implementation at the different steps of the food/feed chain and for the control of the end-products. These methods are:

(i) Time-consuming, whereas techniques that can produce an instantaneous answer are needed;
(ii) Expensive, in a context where appropriate safety and quality control at any crucial link in the food chain require a huge number of analyses to be performed;
(iii) Performed in the laboratory, although the management control needs to be at the production level (on-line measurement) or at the field level (in-field measurement);
(iv) Inflexible and single purpose (one method/one parameter), whereas rapid methods that allow the simultaneous analyses of different compounds are needed;
(v) Sampling dependent, whereas the analysis should be representative of the whole product batch;
(vi) Not always respectful of the environment (toxic reagents), in the context of an international analytical community that is looking for ways of minimising the impact of any actions on the environment or quality of life.

The limitations of the reference methods for food and feed safety and quality control have prompted research teams from public centres, universities and private companies to develop new analytical solutions based on spectroscopic technologies, such as fluorescence spectroscopy, near-infrared spectroscopy (NIR), mid-infrared spectroscopy (MID) and Raman spectroscopy [1–10]. The advantages of spectroscopic techniques are their speed, ease of use, reasonable start-up cost, non-destructiveness and the possibility of implementation on-line or directly in the field. Spectroscopic methods enable a much higher level and frequency of product control, leading to an improved food safety and quality control system. The development of robust and flexible spectroscopic instrumentations adapted for on-line/in-field control of the production chain is well suited for the continuous monitoring of processes from raw materials to finished products. Such systems provide real-time analyses with an increased sample throughput. Other decisive advantages of spectroscopic methods are the ability to determine different factors simultaneously, reduced need to use reagents and reduced sample preparation.

New developments in the spectroscopic area include the development of NIR hyperspectral imaging instruments [11,12]. NIR image analysis is a growing sector in life sciences [13–15]. In agronomy in particular, the various tools now available that enable objective, repeatable, rapid and non-destructive observations to be made are

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easily adapted to the preparation of samples of a very different nature, from the detection of molecular components on a micrometric scale to geographical studies of a particular territory on a kilometric scale. Thanks to image analysis, the conformity of products involved in quality control can be constantly optimised. All these applications contribute to a more precise knowledge of life science mechanisms. Of particular interest from a public health perspective are instruments designed for multispectral or NIR hyperspectral imaging analysis that already play a key role in automatic food and feed inspection [16–22] and will continue to do so. In recent years, NIR hyperspectral imaging has demonstrated its suitability for quality and safety control in the feed sector; it has been used successfully, for example, in the detection of processed animal proteins in feed [23–26], as well as in the cereal sector [27–32] where it enables rapid collection of a multitude of spectra of individual kernels or particles, which is of great interest for laboratories involved in the control of compound feed or cereals. Several applications of NIR hyperspectral imaging relate to the detection of pathogens on plants, as insects [33,34] and fungi [35–40]. Other applications of NIR hyperspectral imaging include the post-harvest handling of fruit and vegetables [41–44], the quality control of meat [45–47] and other agricultural domains [48–50].

Alongside this increased use of spectroscopic techniques, there have also been significant developments in chemometrics. Chemometrics is a chemical discipline that uses mathematics and statistics to design or select optimal experimental procedures, to underline relevant chemical information by analysing chemical data, and to generate knowledge about chemical systems [51]. The main developments have been in i) data reduction, allowing faster data analysis, ii) the construction and management of databanks, iii) the combination of data from different techniques, iv) the development of algorithms to perform appropriate data extraction and exploitation, and v) the interpretation and presentation of the results [52–54]. These improvements have benefited greatly from the incredible development in computer capabilities. For analysts and chemometricians, NIR hyperspectral imaging represents something of a revolution, with hundreds or thousands of spectra (including tens or hundreds of variables) being collected for each sample, instead of the unique average spectrum typically collected using classical spectroscopic instrumentation [17]. NIR image analysis needs the development of very specific chemometric strategies and algorithms in order to get the spatial information from the images and link it to physical or chemical characteristics. There is a growing need, therefore, to combine NIR hyperspectral imaging with recent chemometric developments in order to develop methods for the improvement of the safety and quality of the food and feed chain.

The research objective of this paper is to show that combining NIR hyperspectral imaging spectroscopic techniques with chemometrics can be an elegant solution to set up methods for the improvement of food and feed safety and quality control. In other words, the aim is to achieve major improvements in the ability of new spectroscopic methods to solve food/feed safety and quality analytical challenges in order to provide higher levels of consumer assurance not previously possible. The paper presents two case studies related to quality and safety control in the agricultural sector.

In the first case study, the issue addressed was cereal contamination. Cereals are one of the most important sources of raw material for food and feed in the world. Cereal producers seldom have adequate means to separate cereal grains from various contaminants (harmful or not) that could be accidentally or voluntary included. For instance, in wheat grain one can find such substances as straw, damaged or spoiled grains, grains from other cereals (called also botanical impurities), insects or plastic. Although these impurities are usually less problematic than harmful contaminants, from an economic and technological perspective they can be a great damage problem for cereal producers and transformers. Cereal producers are paid according to the quality of their harvest and will be finally penalised by poor quality batch. Cereal transformers will have additional costs due to implementation of cleaning procedures or will have technological implication of undesirable impurities. For these reasons, this study was limited to looking at diverse contaminants (essentially of vegetable origin) in cereals. The aim was to show the advantages of using NIR hyperspectral imaging spectroscopy and chemometrics in order to integrate them into automatic cereal control procedures at the service of the seed, food and feed sectors.

The second case study focused on the infestation of plants by pathogens. Plant diseases remain one of the major problems in crop production. The easiest and most economical way of ridding plants of parasites is to breed varieties that are resistant to them. Many research programs throughout the world aim at breeding varieties with durable or long-term resistance. The study described here concerns the infestation of sugar beet plants by the cyst nematode, *Heterodera schachtii*, which causes significant yield reduction. In classical breeding programs, the assessment of tolerant and susceptible plants to this cyst nematode is carried out by visually counting the number of cysts in the whole root area using optical microscopy [55]. The aim of the study was to assess, using NIR hyperspectral imaging, the susceptibility of sugar beet plants to nematodes in function of the number of cysts present [56,57].

2. Material and methods

For these studies, two types of instruments were used to obtain the NIR hyperspectral images. The first one was a pushbroom instrument (also called line-scan instrument) and the second was a whiskbroom instrument (also called plane scan instrument). Both instruments can be differentiated on the basis of the orientation of the scanning dimension relative to the two-dimensional spatial sample axes.

For the pushbroom system projects a line of light onto a two-dimensional Focal Plane Array (FPA), and a two-dimensional calibration model (spatial–spectral) is needed to account for variation in sample illumination and instrument throughput. This instrumentation is best suited for remote sensing by aircraft or on-line process measurement because the Y spatial axis might be arbitrarily long. The whiskbroom system positions the measurement camera parallel to the sample surface, obtaining X–Y spatial images with fixed sizes limited by the dimensions (pixels) of the camera detector. NIR hyperspectral images are obtained by modulating the reflected radiation reaching the camera via the use of band pass or tuneable filters positioned in front of the camera [22].

The pushbroom system used was the SWIR XEVA CL 2.5 320 TE4 camera from XENICS using an ImSpector N25E spectrograph that included a cooled, temperature-stabilised Mercury–Cadmium–Telluride (MCT) detector (SPECIM Ltd, Oulu, Finland) combined with a conveyor belt (Burgermetrics SIA, Riga, Latvia). All the images consisted of lines of 320 pixels acquired at 209 wavelength channels: 1100–2400 nm, with a spectral resolution of 6.3 nm and resulting from the averaging of 32 scans. The system and the data treatment were controlled by HyperPro and HyperSee softwares, respectively (BurgerMetrics SIA, Riga, Latvia).

The whiskbroom system used was a MatrixNIR™ Chemical Imaging instrument (Malvern instruments, Analytical Imaging, Columbia, MD, USA)). It is equipped with an InGaAs FPA including 240×320 pixels (76,800 spectra per scan), along with two liquid crystal tuneable filters (LCTF) for wavelength selection. It collects reflectance images in the 900–1700 nm spectral range, with a resolution of 10 nm. Two configurations can be used, depending on the optic and the illumination device installed. The image planes are stacked to form a three-sided matrix, where the first two axes (x and y) define the field of view (FOV), which covers an area between 5 cm^2 and 120 cm^2 depending on the configuration used, and the third axis (z) corresponds to the spectrum at each pixel location in the FOV [19].

3. Case studies

3.1. Case study 1

The first case study sought to detect diverse impurities from different origins in cereals. The samples analysed came from three types of cereal cultures of several origins. In total, 112 samples of wheat, spelt and barley were used in the study. As a first step, for all the samples a crude separation of the cereal grains from the impurities was done using a specific machine [58]. The second step involved a visual and manual separation of the impurities. These contaminants included straw, broken grains, grains from other crops, weed seeds, insects, plastic, stones, pieces of wood and paintings, as well as animal faeces.

For each pure cereal, pure impurity and mixture of cereals with several impurities, images were acquired using the whiskbroom imaging system. A selection of the most representative spectra was made, for each individual image, by using a Kennard and Stone algorithm [59] after applying a mask to remove the background. In such a way, a library for each individual class was obtained. Then, a common spectral database containing 24,000 spectra was constructed and included spectra of cereals (wheat, spelt, barley and rapeseed), cellulose waste (wood and straw), animal contaminants (insects) and other contaminants (paintings, plastic and stones).

All the spectra were pre-processed using smoothing (window = 5), SNV and first derivative Savitzky–Golay (window = 5, polynomial = 2). In order to trace the origin of the materials, including the cereals and the impurities, PCA was applied initially to get some indication of the possible discriminant equations to construct. PCA was able to distinguish easily some groups of impurities, such as insects and stones, but for other groups of impurities it was unable to make a clear distinction between them.

Based on the information obtained from the PCA, discriminant models for the different categories of impurities were created. Due to its powerful discrimination ability compared with other methods, the Quadratic Support Vector Machines (SVM) with an RBF Gaussian kernel was used to construct these models [23,60,61]. The choice of SVM as a classification method was justified by its good performance in all the studies, due mainly to the uniqueness of SVM in tackling pattern recognition problems. For this work, five categories of samples were used to make the discriminant models. The categories were: animal contaminants (i.e. insects), cereals (including wheat, spelt and barley), botanical impurities (it means grains from other cereals like rapeseed), cellulose waste (i.e. wood and straw) and other contaminants (i.e. paintings, plastic and stones). Then, in order to trace the origin of the different materials, a dichotomist classification tree was built based on the five categories, allowing the discrimination of each group of impurities because each node of the tree included a discrimination model constructed using the spectral databank of the five categories (see Fig. 1). By analysing the results of each discriminant model, it was possible to determine whether a category of impurities was present or not, and to estimate its percentage in the sample.

In order to apply SVM, for each node of the dichotomist tree, the spectral dataset was split, after pre-processing, into a calibration set to build the model, a stop set to optimise the SVM parameters and a test set to validate the models using the Kennard and Stone method [59] and their group representativity was demonstrated [63]. Once the optimal parameters selected, the final models were constructed by combining the calibration and the stop sets. Table 1 shows the performances of the models applied in the test sets in terms of confusion matrices, including sensitivity and specificity. Sensitivity is the proportion of spectra detected as positive for one class that actually are positive, whereas the specificity is the proportion of spectra detected as negative for one class that actually are negative. For example, the first equation concerned the discrimination between background and the rest. When applying the results to the test set, 95.48% of the background spectra were correctly detected as background and 98.28% of the other samples were correctly detected as being the rest. In general, correct classification rates higher than 95% were obtained.

To test if the equations worked correctly and could be applied in routine analysis, samples were created by mixing different independent impurities representing those present in the original dataset, and then the different equations were applied. Fig. 2 shows the original image and the different prediction images after the application of

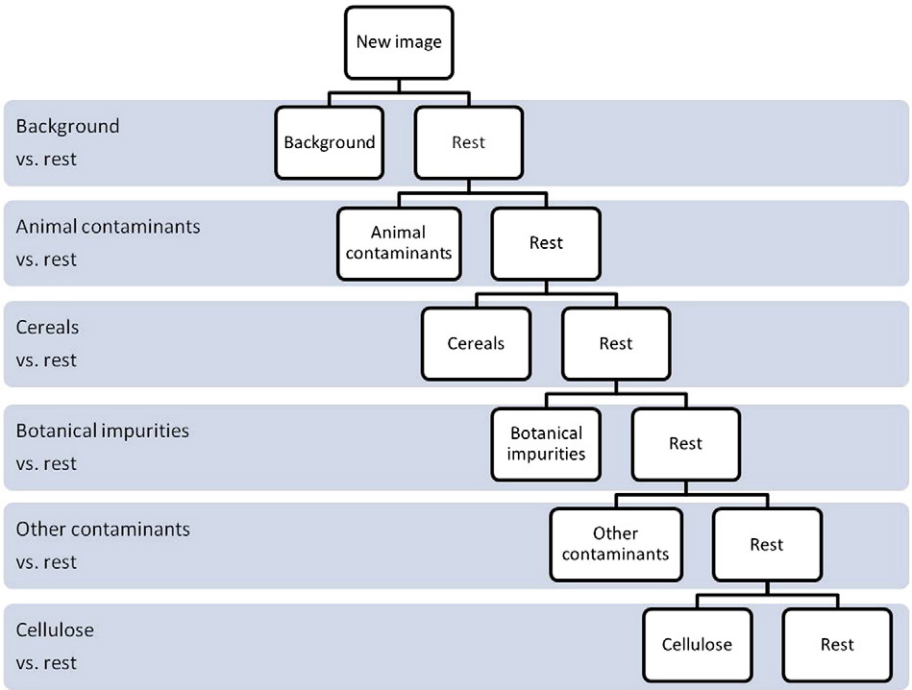

Fig. 1. A dichotomist classification tree including six equations related to background, animal contaminants (insects), cereals (including wheat, spelt and barley), botanical impurities (rapeseed), cellulose waste (wood and straw) and other contaminants (paintings, plastic and stones).

Table 1
First case study: Performances of the models applied to the test sets in terms of sensitivity and specificity.

Detected as		
	Background	Rest
Background	0.955	0.045
Rest	0.017	0.983
	Animal contaminant	Rest
Animal contaminants	0.968	0.032
Rest	0.005	0.995
	Cereals	Rest
Cereals	0.928	0.072
Rest	0.008	0.993
	Botanical impurities	Rest
Botanical impurities	0.967	0.033
Rest	0.001	0.999
	Other contaminants	Rest
Other contaminants	0.980	0.021
Rest	0.003	0.997
	Cellulose waste	Rest
Cellulose waste	0.983	0.017
Rest	0.074	0.926

each of the equations of the classification tree nodes. The pixels detected as cereal are indicated in blue, pixels detected as animal contaminant in red, pixels detected as botanical impurities in yellow, pixels detected as cellulose waste in green and pixels detected as other contaminant in pink. The same colours have been applied in Fig. 3, which shows a plot of the mean spectra of the impurities and cereals.

The first image consisted of grains of wheat, a piece of straw and a grain of maize. The equations detected both the maize and the wheat as cereal and the straw as a cellulose residue. The second image consisted of rapeseeds, insects and wheat grains. All the elements were easily detected by the respective equations. The third image consisted of one piece of painting, two barley grains surrounded by chaffy basal bracts and two grains of wheat. All the samples were easily detected by the respective equations. The barley grains were detected not only by the cereal equation but also by the last equation corresponding to the cellulose content. This can be explained by the fact that roughage (chaff) contains mainly fibre.

The aim of this first case study was to show the great potential of hyperspectral imaging combined with chemometrics for the detection of contaminants in cereals. The system used here was a whiskbroom imaging system, which is not the ideal system to apply in routine analysis by integration into an automatic cereal control procedure. For this, a pushbroom system would be more appropriate as demonstrated in the second case study [40].

3.2. Case study 2

The second case study sought to assess the number of cyst nematodes on sugar beet plants. For this experiment, 20 sugar beet plants with two levels of resistance were grown in a soil support spread on plastic plates and inoculated with juveniles of the Beet Cyst Nematode (*Heterodera schachtii*). Four weeks after inoculation and before NIR hyperspectral imaging, the number of cyst nematodes was visually counted by optical microscopy using a camera system [55], and this was used as a reference. All the plants were then analysed using the pushbroom NIR hyperspectral imaging system at a conveyor belt speed of 2 mm/s.

For detection and possible quantification, a complete spectral library, including spectra from the background (including a water feed strip and a plastic box), soil support, roots and cyst nematodes, was built by selecting around 500 pixels in each region of interest on the images of 10 plants (5 tolerant and 5 susceptible). In total, more than 2000 spectra were used to build the SVM discrimination models. The spectra dataset was preprocessed using smoothing (window = 5), SNV and first derivative Savitzky–Golay (window = 5, polynomial = 2). As for case study 1, for each SVM equation, the spectral dataset was randomly split into a calibration set to build the model, a stop set to optimise the SVM parameters and a test set to validate the models in a ratio of 60, 25 and 15% respectively. A simple PCA projection has been performed in order to check the homogeneity of the three subsets. Once the optimal parameters selected, the final models were constructed by combining the calibration and the stop sets. As in the first case study, a dichotomist classification tree was built in which each node included either a discrimination model constructed using the spectral dataset or a spectral rule based on experience. The equations built were: (1) 'background vs. soil support + root + cyst' aimed at eliminating all the background pixels; (2) 'soil

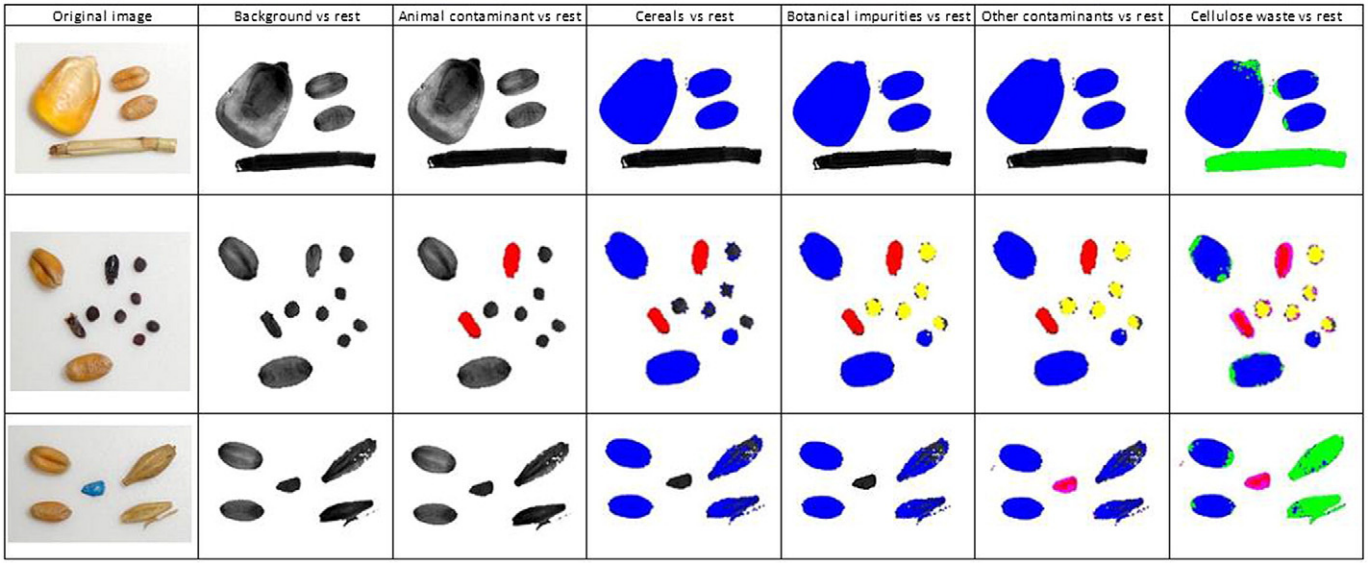

Fig. 2. Three examples of the application of each of the equations of the classification tree nodes. Each example includes the original image and the prediction images. Pixels are coloured as follows: detected as cereal are indicated in blue, detected as animal contaminant in red, detected as botanical impurities in yellow, detected as cellulose waste in green and detected as other contaminant in pink.

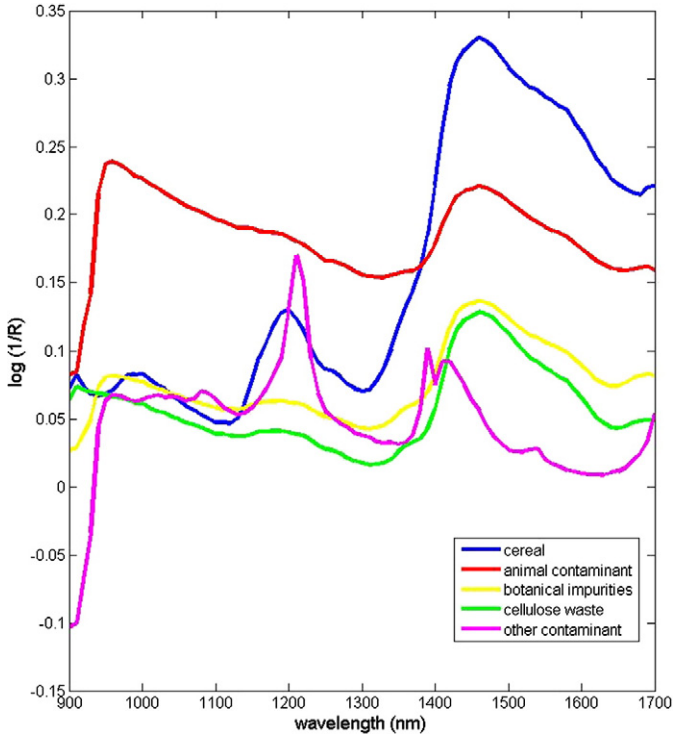

Fig. 3. Mean spectra of several impurities and cereals. Spectra are coloured as follows: cereal in blue, animal contaminant in red, botanical impurities in yellow, cellulose waste in green and other contaminant in pink.

support vs. root + cyst' aimed at removing all pixels related to the soil support; and (3) and 'root vs. cyst' aimed at detecting the presence of cysts. Table 2 shows the performance of the models applied to the test sets. The complete dichotomist classification tree included the following steps (visually represented in Fig. 4):

(1) Detection of pixels in the image, showing a higher absorbance around 1690 nm than around 1970 nm, corresponding to the conveyor belt (indicated in black in the original image in Fig. 4a and white in Fig. 4b after removing the pixels);
(2) Detection of pixels in the image, detected as soil support, roots or cysts by the SVM model 'background vs. soil support + root + cyst' (indicated in grey in Fig. 4c);
(3) Detection of pixels in the image, detected as roots or cysts by the SVM model 'soil support vs. root + cyst' (indicated in grey in Fig. 4d);
(4) Detection of pixels in the image, detected as cysts by the SVM model 'root vs. cyst' (indicated in grey in Fig. 4e);
(5) Removal of the pixels classified as outliers according to rules based on the comparison of absorbance at several wavelengths; the cysts showing a lower absorbance around 1734 nm than around 1715 and 1765 nm (Fig. 4f);
(6) Application of the Density-Based Spatial Clustering of Applications with Noise (DBSCAN) method [62] to study the neighbourhood of the pixels detected as cysts in step (5). Using this technique, pixels within 1 pixel of each other and with a minimum of two neighbour pixels were placed in a single class and identified as a cyst (indicated in blue in Fig. 5b). Pixels that did not meet these conditions were identified as outliers.

Table 2
Second case study: Performances of the models applied to the test sets in terms of sensitivity and specificity.

Detected as		
	Background	Rest
Background	1	0
Rest	0	1
	Soil support	Rest
Soil support	1	0
Rest	0.024	0.976
	Root	Cyst
Root	1	0
Cyst	0.015	0.985

Once the models were constructed and validated, the complete discrimination tree, including the three equations and the spectral rules, was applied successively to all the pixels in the images of the 20 plants in order to estimate the number of pixels detected as cysts by surface unit. Fig. 5 shows the results obtained for a susceptible plant. The images show the cysts coloured in red or blue after detection using optical microscopy (Fig. 5a) or pushbroom imaging (Fig. 5b), respectively.

Based on the results from the 20 plants, a determination coefficient of 0.71 was calculated between the number of cysts counted on the roots using optical microscopy and the number of pixels recognised as nematode cysts using the NIR hyperspectral imaging method. However, most of the tolerant plants and some susceptible plants were underestimated as indicated by the regression parameters (slope of 1.22 and offset of 26.16). It has to be noted that the underestimation of the cysts number is issued from the calculating process: one cluster is considered as 1 cyst whereas some clusters identified using the DBSCAN method can contain more than 1 cyst. However, this does not affect the main issue of this study which is to assess the susceptibility of sugar beet plants to nematodes in function of the number of cysts present. An average of 40 and 69 for the tolerant and susceptible plants, respectively, was counted using classical microscopy, whereas for the NIR hyperspectral imaging method these predicted values were 21 and 58 cysts, respectively. Despite the underestimation by NIR hyperspectral imaging, the obtained correlation could make possible the development of robust and reliable discrimination models between tolerant and susceptible plants.

A similar study was performed with the whiskbroom NIR hyperspectral imaging system. A determination coefficient of 0.65 was calculated between the number of cysts counted on the roots using optical microscopy and the number of pixels recognised as nematode cysts using the whiskbroom method. The lower correlation obtained with this instrument was because the whiskbroom NIR hyperspectral imaging system does not allow a complete image of the sample to be obtained, as was the case with the NIR pushbroom system, thereby adding a new source of uncertainty to the measurement. The advantages of the pushbroom system compared with the whiskbroom system are that the whole plate can be analysed once, the wavelength range is larger and the acquisition time is lower.

4. Conclusion

The first case study demonstrated that the use of NIR hyperspectral imaging combined with chemometric tools is potentially interesting for the simultaneous detection in cereals of a series of contaminants from different origin. This kind of technology contributes to improving the quality of products in the food and feed processing industry. In addition, it represents a huge increase of the speed of measurement and therefore a reduction in the costs of analyses. The second study showed the potential of the NIR hyperspectral imaging for discriminating cysts from the roots and soil support in sugar beet roots, and for quantifying the number of cysts. This technique could help plant breeders in assessing germplasm tolerance and susceptibility to pests and diseases and in quantifying the level of damage or resistance to the pathogens or insects. Besides the results shown in this work, the methodologies proposed here could also be applied in numerous other domains in the

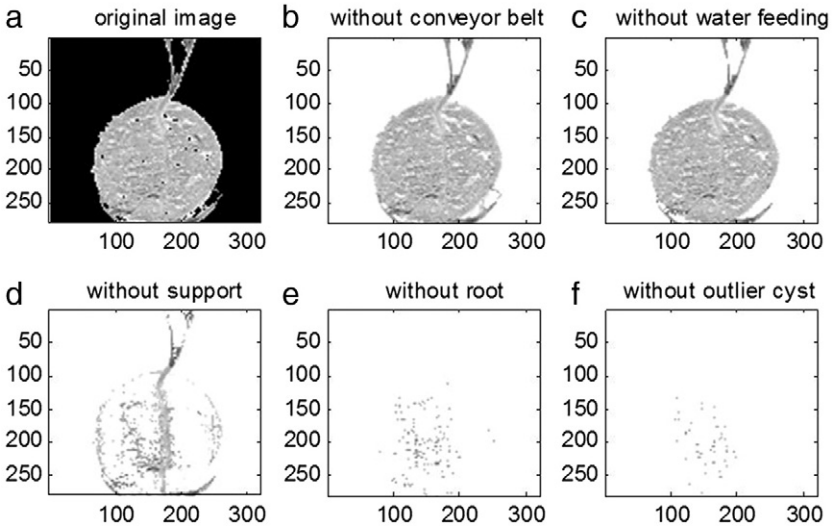

Fig. 4. Cyst detection on sugar beet plant using the SVM model displaying one image capture of 270∗320 pixels for a) the NIR hyperspectral image at 1000 nm, b) the image 'a' after removing the pixels detected as spectra belonging to the conveyor belt, c) the image 'b' after removing the pixels detected as spectra belonging to the plastic box and the water feeding strip, d) the image 'c' after removing the pixels detected as spectra belonging to the soil support, e) the image 'd' after removing the pixels detected as spectra belonging to the root, and f) the image 'e' after removing the pixels detected as outlier cysts.

selection of ingredients at the beginning/end of a production chain. NIR hyperspectral imaging can also be used in large number of researches in the agricultural sector, as the qualitative and quantitative identification of animal particles in feed, the detection and quantification of different contaminants in food/feed, seed control in factories or disease control in a breeding process among others. These studies need always to be combined with powerful chemometric tools in order to create a complete procedure allowing dealing with on-line/off-line prediction in a production system for the quality control of agricultural products.

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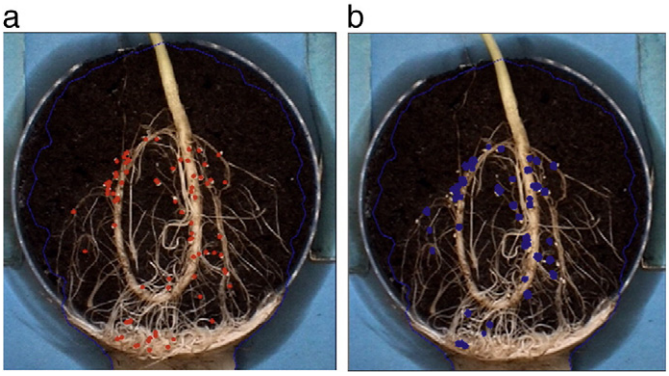

Fig. 5. Nematode cyst number counted using (a) optical microscopy (cysts coloured in red) and (b) the NIR hyperspectral imaging system (cysts coloured in blue) on a susceptible sugar beet plant.

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