Development of near infrared spectroscopy methods for the detection of ergot in cereal flour

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Introduction

Since decades, near infrared (NIR) spectroscopy is widely used in the food and feed sectors to implement rapidly, inexpensive and efficient control tools for the quality of products. In the 2000s, in the framework of the BSE (bovine spongiform encephalopathy) crisis, NIR microscopy (NIRM) and NIR hyperspectral imaging have been introduced for the authentication of feed ingredients and the detection of animal ingredient particles in feedstuffs [1]. More recently, this technology was investigated to detect undesirable and toxic contaminants in cereals intended for the food and feed sectors. In particular, methods were developed for the on-line detection and quantification of ergot bodies formed by the fungus *Claviceps purpurea*, which contains toxic alkaloids [2]. This work aims to assess NIR microscopy (in transmission mode) and NIR hyperspectral imaging (in reflection mode) in comparison to NIR spectroscopy and light microscopy to detect particles of ergot bodies in cereal flour.

Reference methods

Zeiss: Light microscope

Visual observations combined with confirmation by light microscopy are the reference method for ergot contamination in grains. Protocol was adapted to be used on grounded samples using Sudan red coloration. Chromatographic methods are the reference methods to assess the alkaloid content. The Arvalis set of 13 samples containing alkaloids was analysed using LC-MSMS.

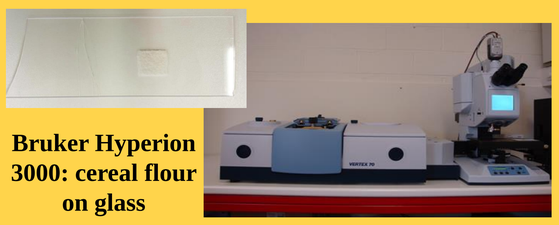
NIR spectroscopy

Foss XDS: ring cup with cereal flour

Alternative methods that are rapid, non destructive, requiring no sample preparation and no solvent are needed. Previous studies [3] have proved that near infrared (NIR) spectroscopy combined with sampling accessories seems to be an interesting technique for the determination of contaminant in grains.

NIR microscopy

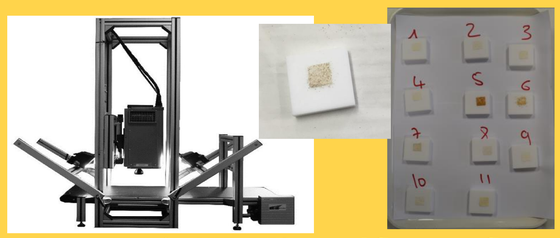
FT-NIR microscope Hyperion 3000 (Bruker) with motorized platform was also used for this purpose. Near spectra (1113-2521 nm) were recorded in transmission mode with 8 scans by particle and a window of 50 µm. A mapping of 400 spectra was recorded per sample with an aperture of 8 mm.



Bruker Hyperion 3000: cereal flour on glass

NIR hyperspectral imaging

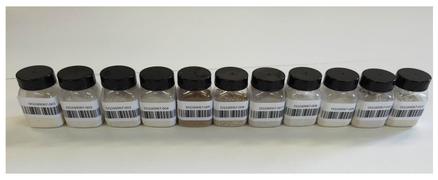
NIR hyperspectral imaging system with a conveyor belt (Burgermetrics) was used as well. Near spectra (1118-2425 nm) were recorded in reflection mode with 32 scans by pixel (30 µm x 30 µm). An image of 320 * 400 spectra was recorded per sample.



Burgermetrics NIR HIS: cereal flour on teflon

Analyses and Results

For this study, ground ergot bodies samples from different sources (wheat and rye) and cereal flour samples from different species (wheat, rye) and different composition (with or without germ and bran) as well as mixtures of both from 1 % to 50 % of ergot (CRA-W samples set) and 10 to 57884 ppb of alkaloids (Arvalis sample set) were analysed. Principal component analysis (PCA) and partial least squares discriminant analysis (PLSDA) models were developed and applied on the 3 sets of spectra: NIRS, NIRM mapping and spectral images in order to detect the ergot bodies particles.



Light microscopy

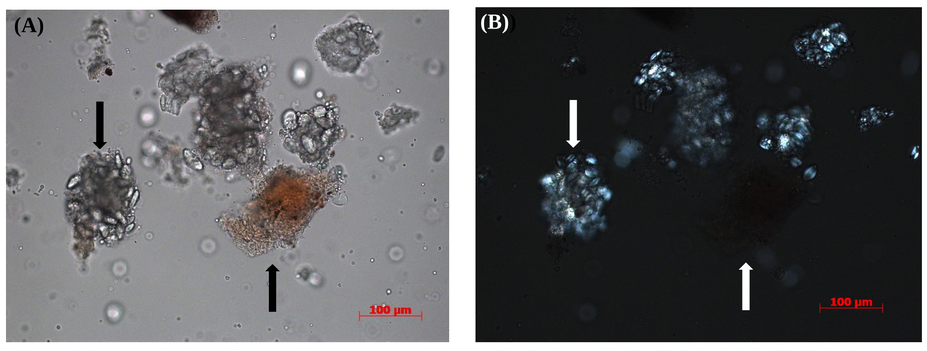


Fig 1: Mixture of white flour with pure ergot colored with Sudan red. (A) Bright field, (B) Polarization. A fragment of sclerotia (up arrow) colored in light background, starch aggregate (down arrow).

➡ Ergot detection based on Sudan red coloration combined with polarized light analysis and hyphae structure observation against plant structure.

NIR spectroscopy

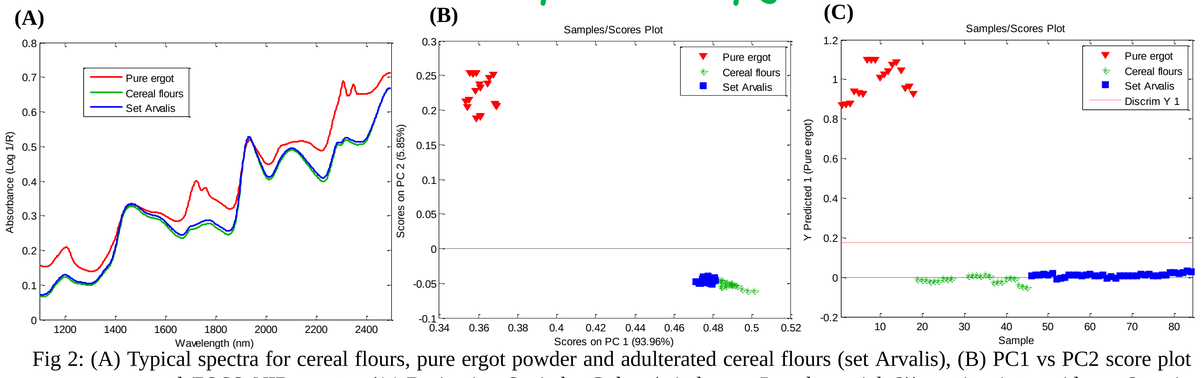


Fig 2: (A) Typical spectra for cereal flours, pure ergot powder and adulterated cereal flours (set Arvalis), (B) PC1 vs PC2 score plot on pre-processed FOSS NIR spectra (1st Derivative Savitzky-Golay (window = 5, polynomial=2)) putting into evidence 2 main groups: ergot / other samples, (C) Samples predicted as ergot or cereal flours using PLS-DA model applied on NIR spectra.

➡ No detection of adulterated samples based on PLSDA models applied on NIR spectra of adulterated samples (3 spectra / sample).

NIR microscopy

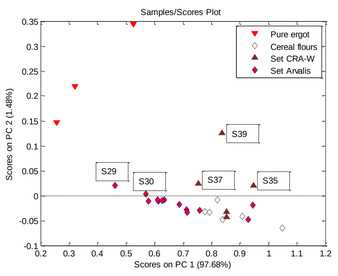


Fig 3: PCA applied on mean spectra showing 3 groups: Pure ergot, cereal flours and adulterated flours.

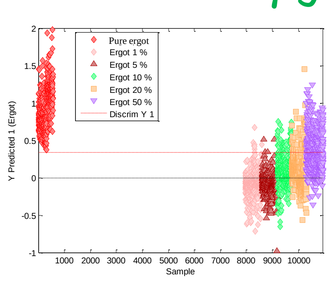


Fig 4: Particles predicted as ergot according to the adulteration level (CRA-W samples set).

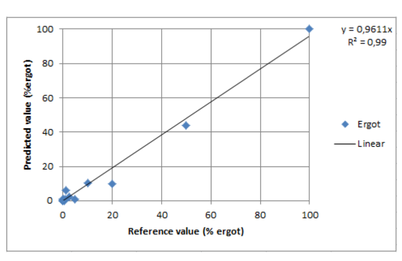


Fig 5: Correlation between the reference values expressed in % ergot on full samples set and the predicted values using PLS-DA model applied on NIRM spectra.

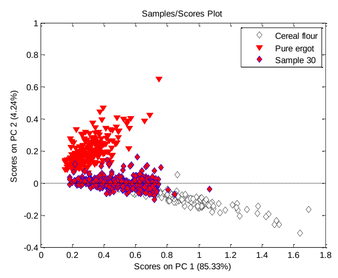


Fig 6: PCA applied on all spectra showing some particles of adulterated flours closed to particles of pure ergot.

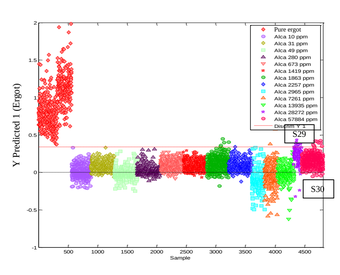


Fig 7: Particles predicted as ergot according to the adulteration level (Arvalis sample set).

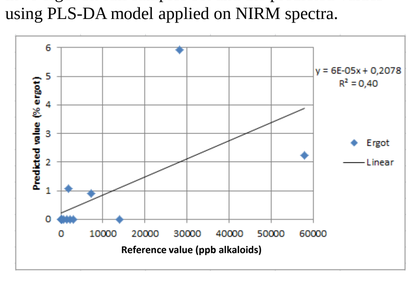


Fig 8: Correlation between the reference values expressed in ppb of alkaloids on Arvalis sample set and the predicted values using PLS-DA model applied on NIRM spectra.

NIR hyperspectral imaging

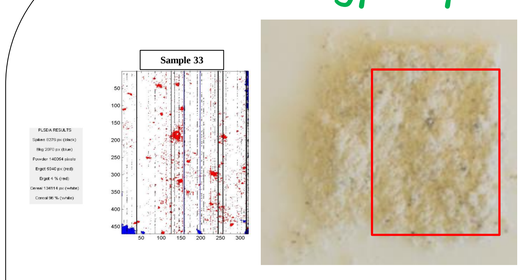


Fig 9: Predicted images and RGB picture showing ergot particles (red) in a wheat flour (white) sample adulterated with 5 % of ergot powder.

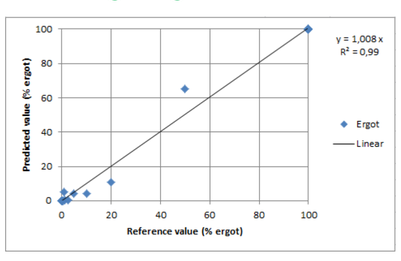


Fig 10: Correlation between the reference values expressed in % ergot on full samples set and the predicted values using PLS-DA model applied on NIR hyperspectral images.

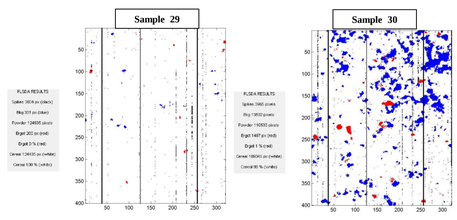


Fig 11: Predicted images showing ergot particles (red) in a wheat flour (white) sample adulterated at 28272 and 57884 ppb of alkaloids, respectively sample 29 and sample 30.

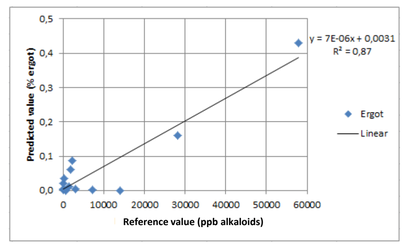


Fig 12: Correlation between the reference values expressed in ppb of alkaloids on Arvalis sample set and the predicted values using PLS-DA model applied on NIR hyperspectral images.

➡ Detection of ergot particles based on PLSDA models applied on NIRM spectra of adulterated samples (400 spectra / sample).

➡ Detection of ergot particles based on PLSDA models applied on NIR hyperspectral images of adulterated samples (120000 spectra / sample).

Conclusions

This study showed the feasability of light microscopy by Sudan red coloration to detect ergot in cereal flour as well as the potential of NIRM and NIR hyperspectral imaging combined with chemometrics to propose alternative solutions to discriminate the ergot bodies particles from cereal flour.

Acknowledgements

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